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Internesting Diving Behavior and Population Structure of Hawksbill Sea Turtles (Eretmochelys imbricata) on St. Croix, USVI

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INTERNESTING DIVING BEHAVIOR AND POPULATION STRUCTURE OF HAWKSBILL SEA TURTLES (*Eretmochelys imbricata*) ON ST. CROIX, USVI

A Thesis

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Hill, Jacob E. M.S., Purdue University, August 2014. Internesting Diving Behavior and Population Structure of Hawksbill Sea Turtles (*Eretmochelys imbricata*) on St. Croix, USVI. Major Professor: Frank V. Paladino.

Hawksbill sea turtles (*Eretmochelys imbricata*) are distributed circumtropically and populations in many locations have been severely depleted. Developing management plans for this species is hindered by major gaps in knowledge concerning habitat use, behavior, and population structure. This study addresses these knowledge gaps for hawksbill sea turtles nesting at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands. Specifically, I will focus on research priorities identified by the US Fish and Wildlife Service in the Recovery Plan for Hawksbill Sea Turtles in the US Caribbean Sea.

The first chapter addresses Recovery Plan Objective 111: *Identify Important Nesting Beaches*. Three seasons of nighttime monitoring were conducted on Sandy Point to tag and identify nesting hawksbills and to document all nesting activities on the beach. A total of 78 individual hawksbills were identified, with an average of 26 nesting females per season and a range of 100-500 nesting activities per year, which puts Sandy Point in the top 5% of hawksbill beaches in the Wider Caribbean based on these annual

numbers of turtle activities. These data establish Sandy Point as a major rookery in the Eastern Caribbean and support its designation as an index beach for future monitoring of hawksbill nesting trends.

In the second chapter, Objective 2112 is addressed: *Determine adult internesting movements*. Time-depth recorders were deployed on nesting hawksbills to examine internesting diving behavior. Hawksbill turtles spent the majority of the internesting interval relatively inactive, with long dives to a constant depth and short surface intervals. This behavior suggests individuals are resting on the seafloor in a localized internesting residence area. The depth utilized during this time showed pronounced individual variations, with some turtles remaining in shallow water less than 5 meters deep, while others consistently resided in waters 30 meters in depth. In the few days before returning to nest, dive depth for all internesting turtles became much deeper, with two turtles attaining maximum dive depths of 84.5 and 94.6 meters, which are the deepest recorded dives for hawksbills during the internesting interval. These extremely deep dives were possible because the water column is very deep close to Sandy Point due to the narrow continental shelf, and in addition the water temperature does not fall below 24.5°C in the top 100 meters of the water column in these tropical waters. Such conditions permit long bouts of deep diving that are not possible at other locations for the relatively smaller hawksbills. These results demonstrate that when water temperature is not a limiting factor, internesting hawksbill sea turtles can dive up to 100 meters to locate the seafloor just prior to an emergence and adjacent to the nesting beach.

Lastly, in Chapter 3 I address Objective 217: *Determine the genetic relationships among Caribbean hawksbill nesting populations*. Tissue samples were collected from nesting hawksbills to sequence a control region of mitochondrial DNA. Haplotype profiles from Sandy Point were then compared to previously published haplotype data from other rookeries across the Caribbean. This mitochondrial DNA analysis showed that Sandy Point hawksbills are genetically distinct from every other rookery, including Buck Island Reef National Monument, which is also part of the St. Croix complex of islands and within 40 kilometers of Sandy Point. This genetic differentiation is supported by mark-recapture data, as none of the 78 nesting turtles identified had ever been encountered on nearby Buck Island. These population demographic findings demonstrate that St. Croix has two genetically distinct nesting populations of hawksbill sea turtles. As a result, management plans should consider these beaches separately in order to assess the unique threats facing each site.

Overall, this study provides much needed insight into the biology and population structure of hawksbill sea turtles in the US Virgin Islands. These results provide a foundation on which to develop management plans for hawksbills, while identifying areas of research that should be a priority in the future.

CHAPTER 1

MONITORING OF HAWKSBILL SEA TURTLES AT SANDY POINT NATIONAL WILDLIFE REFUGE, ST. CROIX

Introduction

The conservation and management of long-lived, wide-ranging megafauna poses many challenges (Clark et al. 1996). This holds especially true for marine turtles, which are slow to mature and often undergo vast migrations during the course of their lives (Heppell et al. 2003). The hawksbill turtle (*Eretmochelys imbricata*) inhabits tropical marine waters throughout the Atlantic, Pacific, and Indian Oceans (Witzell 1983). They may be found in bays and lagoons (Plotkin 2003) but are most often associated with coral reefs, where they feed predominately on sponges (Meylan 1988). By selectively feeding on sponges, hawksbills alter reef structure, leading to an increase in coral reef biodiversity (León and Bjorndal 2002). In addition to this important ecological role on coral reefs and tropical coral habitats, hawksbills, like all sea turtle species, are valued for their use in ecotourism, for their cultural significance, and as symbols of marine and environmental conservation (Campbell 2003).

Despite the hawksbill's widespread importance and significance, global hawksbill populations have declined by more than 80% over the past century (Meylan and

Donnelly 1999). In addition to the threats faced by all sea turtle species (reviewed in Heppell et al. 2003), hawksbill populations have long been exploited for their shell, which is considered a precious commodity on par with ivory, gold and gems (Meylan and Donnelly 1999). The trade in hawksbill shell dates back to the 15th century B.C. and millions of hawksbills have been slaughtered as part of this trade, resulting in the longest and most sustained exploitation of any sea turtle species (Parsons 1972). Due to this combination of threats, hawksbills are listed as critically endangered on the IUCN Red List of Threatened Animals (Meylan and Donnelly 1999, IUCN 2014). Many nesting populations are greatly diminished from historic levels and under the current level of threats, populations will likely continue to dwindle (Meylan and Donnelly 1999).

For a critically endangered species like the hawksbill, determining population size is important for monitoring trends and for identifying particular areas of conservation importance. Such data can then be used to focus efforts in order to protect the areas that face the greatest threats. For sea turtles, numbers of nesting females can be used to provide insight into population trends (Witherington et al. 2009). This is a common method of examining population numbers because it is the only time adult sea turtles can be encountered on land, and the tracks left by turtles on the beach can still be documented even if the turtle is not observed (Schroeder and Murphy 1999).

Hawksbill sea turtle nesting in the wider Caribbean is generally diffuse and occurs in low densities across small isolated beaches (Piniak and Eckert 2011). For example, the island of St. Croix contains over 31 separate beaches that have been identified as hawksbill nesting sites, but less than 10 nests per year are laid on the

majority of these beaches (Mackay 2006). Such low density of nesting makes it logistically difficult to regularly access so many beaches in order to quantify nesting activities for hawksbills. As a result, there is a paucity of data regarding numbers of nesting turtles in many beaches in the Caribbean (Piniak and Eckert 2011).

Sandy Point National Wildlife Refuge (17.6800074 N, -64.9031066 W) is located on the southwest peninsula of St. Croix, part of the US Virgin Islands (Figure 1.1, 1.2, 1.3). The refuge contains the longest stretch of continuous beach in the US Virgin Islands and has been protected by the US Fish and Wildlife Service since 1984, which offers a unique opportunity to study hawksbill nesting in the East Caribbean (Evans 2010). Sandy Point is listed as critical habitat for the leatherback sea turtle (*Dermochelys coriacea*) (Meibohm 1979) and a significant amount of USFWS resources have been devoted to studying leatherbacks at this beach, making it the longest continuously running saturation tagging project of leatherbacks in the world (Dutton et al. 2005). Despite the funding that has gone in to studying leatherbacks, hawksbill nesting has been virtually ignored and has been limited to sporadic and opportunistic tagging of nesting females during the leatherback season. The lack of thorough and consistent monitoring for this critically endangered species on Sandy Point, which is part of a USFWS protected refuge means the relative importance of this beach as a hawksbill nesting habitat is unknown.

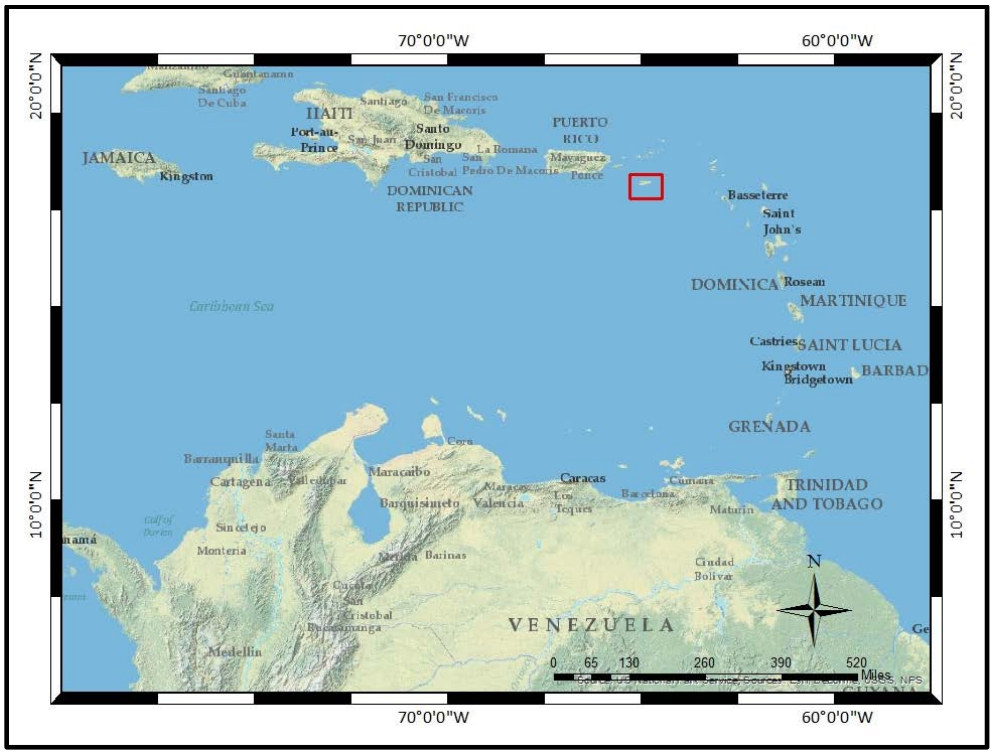


Figure 1.1: Map of the Caribbean. Red box highlights the location of St. Croix.

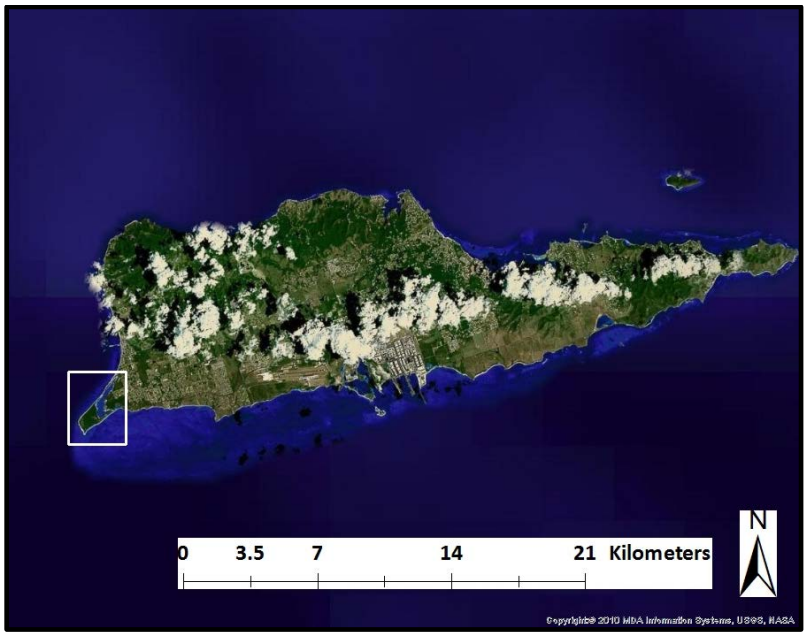


Figure 1.2: Map of St. Croix. Box highlights the location of Sandy Point.

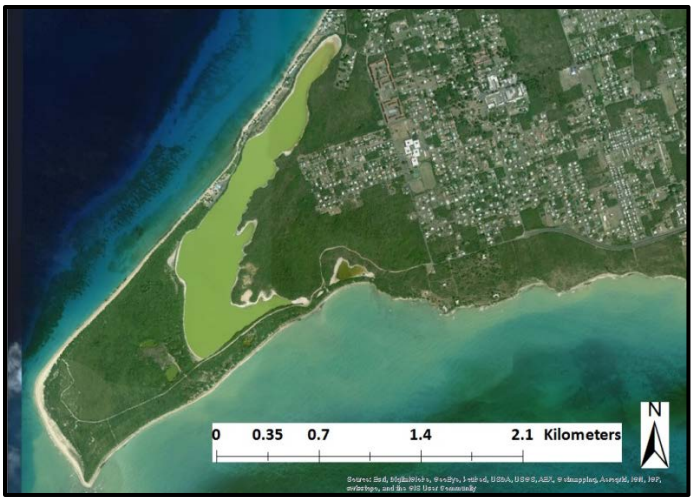


Figure 1.3: Sandy Point National Wildlife Refuge.

Three seasons of nighttime monitoring were conducted to encounter nesting hawksbill turtles on Sandy Point and to document every nesting activity. The goal of this study was to determine the annual number of nesting hawksbills on Sandy Point and to quantify the total number of nesting activities in order to develop a baseline for future monitoring. The objective was to analyze the size of this nesting population in the context of hawksbill nesting in the Eastern Caribbean. Identifying important beaches for nesting is considered a priority by the US Fish and Wildlife Service in the Recovery Plan for Hawksbill Sea Turtles in the US Caribbean Sea (NMFS 1993).

Methods

Tagging

Nightly patrols of the central portion of the beach were conducted to encounter all nesting hawksbills. The times and frequency of patrols varied between the years, as

did the area surveyed, due to varying levels of personnel to assist in data collection. Effort was focused on patrolling the areas of beach with the highest density of nesting. In 2011, 3.2 km were patrolled every night August 15th-October 14th from 20:00-5:00. In 2012, 2.5 km were surveyed six nights each week July 11th-August 31st from 20:00-3:00. In 2013, the same area of beach patrolled in 2012 was patrolled six nights each week May 1st-September 24th 20:00-1:00.

Turtles were approached and tagged only during oviposition. A PIT tag was inserted into the left shoulder (1.4A) and a metal Inconel tags was applied to the right front flipper (Figure 1.4 B). In cases where turtles did not successfully lay eggs, they were checked for tags as they returned to the water. In 2011, untagged individuals were given a flipper tag while departing. However, this procedure was later determined by refuge personnel to be unnecessarily invasive, so in the subsequent two years, tags were only applied during oviposition.

(A) (B)

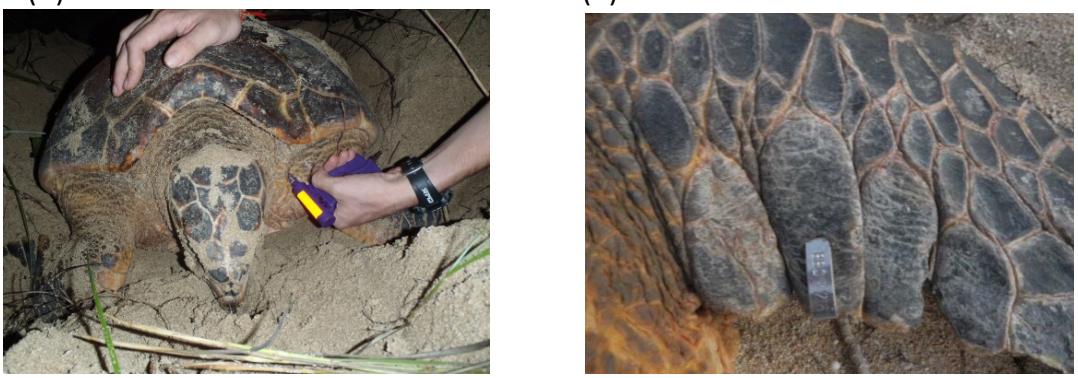
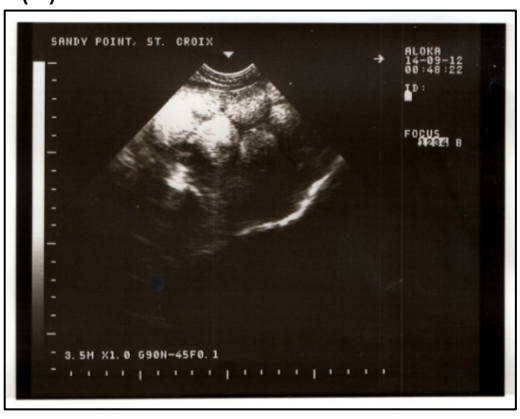


Figure 1.4: (A) Application of PIT tag and (B) flipper tag on right front flipper.

Ultrasonography

In 2012, ultrasonography was employed to examine the reproductive status of nesting turtles. Ovipositing turtles were scanned using a portable, real-time ultrasound scanner (Aloka SSD-500) with a 3.5 MHz linear transducer to examine their ovaries and determine reproductive status. Ovaries were scanned by placing the ultrasound in the inguinal region cranial to the hind limb (Rostal et al. 1990, Blanco et al. 2012). If there were significant numbers of unshelled eggs in the ovary or toward the oviduct or developing follicles toward the end of laying a clutch (Figure 1.5A), the turtle was classified as a candidate to lay a subsequent clutch during the season. However, if the ovaries were depleted, (Figure 1.5B) the turtle was probably laying her final nest of the season and not expected to return.

(A)



(B)

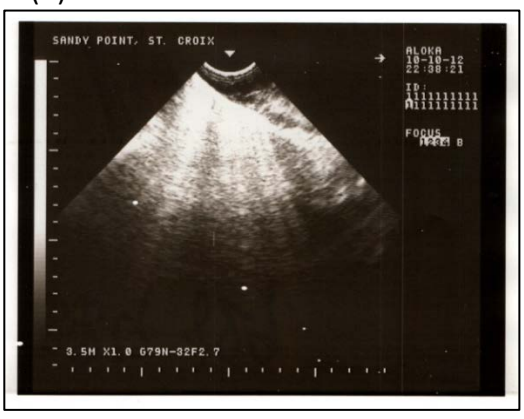


Figure 1.5: (A) Turtle with developing follicles and (B) without developing follicles.

Activity Documentation

In addition to encountering nesting turtles, I also documented every turtle activity via its track on the beach. Activities were classified as one of the following: confirmed lay (eggs seen); probable lay (eggs not seen, but likely deposited); or false crawl (eggs not deposited). Daytime patrols were carried out daily across the entire refuge and activities were also classified in the same manner.

Estimated Clutch Frequency

Because the number of confirmed nests by each turtle was relatively low, I calculated an estimated clutch frequency to predict the number of nests laid by each turtle (Johnson and Ehrhart 1996). To do this, I assumed an internesting interval of 14 days based on those reported from previous studies of Caribbean hawksbills (Beggs et al. 2007, Walcott et al. 2013). Thus, if the number of days between two encounters was 28 days or more, it was assumed the turtle had deposited an unobserved nest between these encounters. The number of nests laid was determined by dividing the number of days between encounters by 14. Also, if a non-nesting encounter was documented 14 days before or after a confirmed nest, it was considered a second nest, and if two non-nesting encounters occurred 14 days apart, it was considered two separate nesting events. Because sample sizes were small per season (n=19-37), I pooled together the clutch frequency data from all three seasons.

Results

I identified 37 unique individuals in 2011, 19 in 2012, and 25 in 2013. Five individuals were seen in both 2011 and 2013. Four individuals were tagged on Sandy Point prior to 2011. I documented between 203 and 550hawksbill activities across the three seasons (Figure 1.6). The average internesting interval was 15.5 days. The estimated clutch frequency was 1.69 ± 1.23 (range 1-5; Figure 1.7). The majority (67%) of individuals were only observed once.

In 2012, I performed ultrasonography on 13 turtles. Two of the turtles were laying their last nest of the season, while 11 had developing follicles. Of these 11, 2 returned to nest again, while the remaining nine were not documented laying subsequent nests.

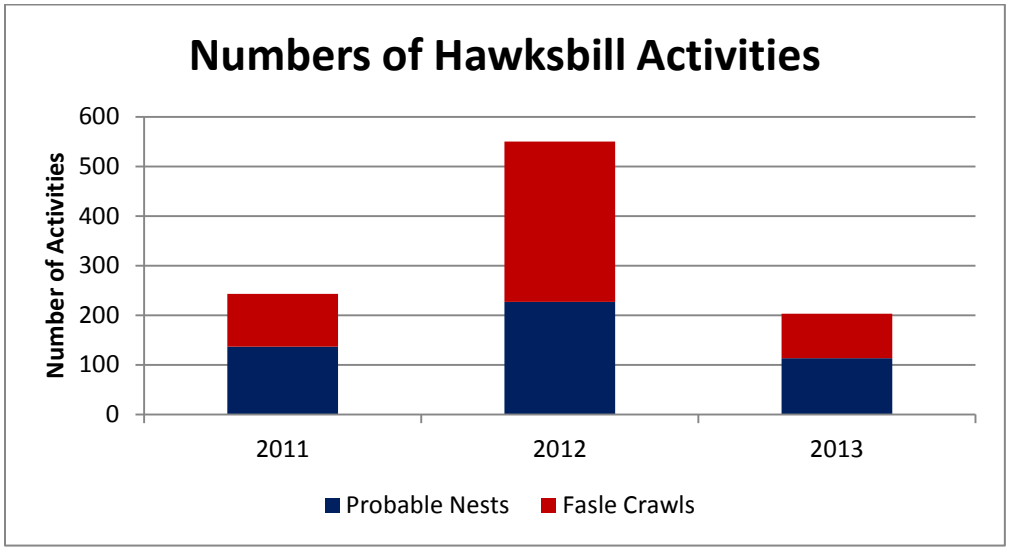


Figure 1.6: Yearly numbers of hawksbill activities. Confirmed nests and probable lays are combined as “probable nests.”

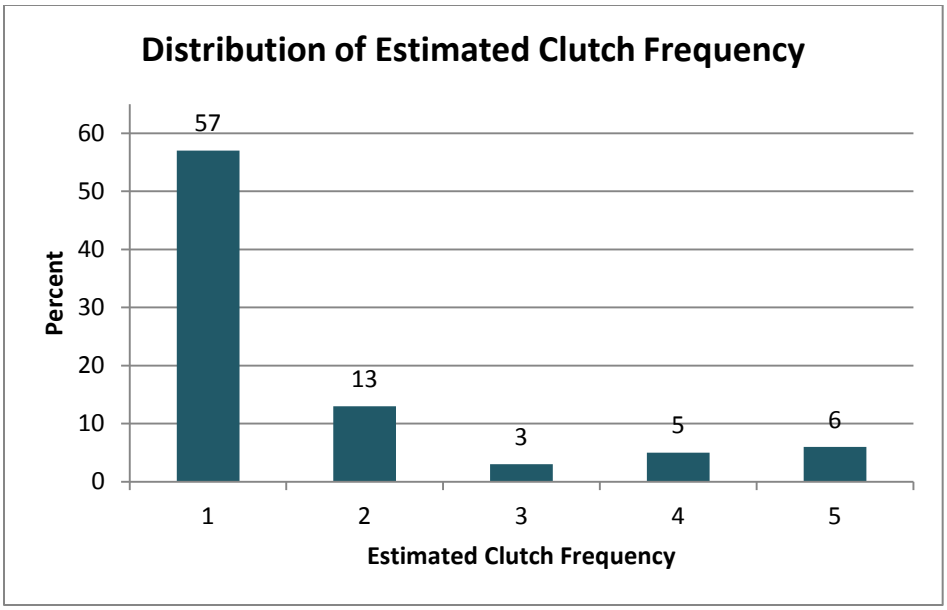


Figure 1.7: Percentage of turtles exhibiting different clutch frequencies.

Discussion

The majority of hawksbills in this study were only encountered once, resulting in an estimated clutch frequency (ECF) of 1.69. In contrast, in studies at other nesting sites, ECF has been as high as 4 or 5 (Beggs et al. 2007). ECF in this study is therefore likely an underestimation and is consistent with other observations of hawksbill nesting at beaches on the east end of St. Croix, in which researchers found that the majority of turtles were only seen once and that observing the same individual on more than one occasion was extremely rare (Mackay 2005, 2006). This trend has also been observed previously on nighttime patrols during leatherback season. For example, in 2006, 16 individual hawksbills laid 20 nests (Garner et al. 2006), and in 2007, 10 individuals laid 16 nests (Garner and Garner 2007). In these seasons, monitoring ended on August 15th

in 2006 and on August 31st in 2007. As such, patrols may not have encompassed all of the hawksbill nesting season. However, since July was identified as the peak nesting season, it is probable that many of the turtles would have been nesting during this time. If a turtle nests on average every 15 days, a turtle that started nesting in early July should have been seen at least 3 times by the time monitoring ended in mid-August if a similar ECF to other hawksbill nesting populations is expected.

The low encounter rate for these turtles is likely because they are dispersing their nests across multiple beaches, showing low nest-site fidelity. This is supported by the ultrasonography data, which showed several turtles with additional clutches of eggs to lay in the season were not seen again. Furthermore, there were cases in which two successive encounters of the same turtle spanned more than a month, which indicated that the turtle may have nested somewhere else during this period. In 2011, the longest interval between successive observations of a turtle was 47 days, which was enough time for the turtle to have laid 2 clutches.

The low site fidelity of hawksbills is possible due to abundant suitable nesting habitat around St. Croix. The south shore of the island is lined with beaches where turtles could successfully nest. At other nesting sites, suitable nesting habitat may be spatially constricted to one place, which results in higher repeatability of nesting locations.

The dispersal of nests may also be adaptive. Sandy Point is host to a large nesting population of leatherbacks and is typical of a leatherback nesting beach in that it is unobstructed by offshore reef and thus is exposed to high rates of erosion (Eckert

1987). As a result, each year an estimated 45-60% of leatherback nests are naturally washed away and lost (Eckert 1987). Also, without an offshore reef as protection, the beach is prone to drastic changes in shoreline due to storms, with storm events sometimes depositing several feet of sand on top of nests, making them unviable (Valiulis 2012). A greater dispersal of nests by individual nesters occurs on beaches subject to higher rates of environmental unpredictability, as a way to ensure that at least some nests will hatch (Eckert 1987). In contrast, nests laid on beaches that are stable have a higher likelihood of hatching across multiple areas of the beach, which means that there is less pressure to scatter nests, resulting in higher nest site repeatability (Kamel and Mrosovsky 2005). While hawksbills generally tend to nest on stable beaches (Kamel and Mrosovsky 2005), patterns of erosion on Sandy Point are spatially and temporally unpredictable, with different areas of the beach eroding in different years (Eckert 1987). As a result, hawksbills may scatter their nests across Sandy Point and other nearby beaches as a way to ensure maximum hatchling production in this highly dynamic environment.

To compare the amount of nesting that occurs on Sandy Point with other beaches in the Caribbean, I used the total number hawksbill tracks. Comparisons using numbers of nesting females is difficult because many places do not conduct nighttime monitoring to identify the numbers of nesting turtles (Piniak and Eckert 2011). Using numbers of nests laid can also be problematic because of differences in the criteria used to classify a nesting event when the clutch is not located. Fortunately, there has been a Caribbean-wide documentation of turtle activities across most nesting beaches, which

provides a means by which to compare Sandy Point to other beaches (Piniak and Eckert 2011). There were between 100-500 hawksbill activities yr^{-1} in 2011 and 2013, while nesting in 2012 exceeded this range. In the majority (52%) of beaches that documented nesting by hawksbills, there was less than 25 activities yr^{-1}. In rankings of numbers of annual hawksbill nesting activities, Sandy Point falls in the top 36 out of 817 beaches that reported nesting by hawksbills, making it in the top 5% (Piniak and Eckert 2011). The relative amount of nesting that occurs by hawksbills on Sandy Point becomes more pronounced when examining it in the context of nesting in the Eastern Caribbean. Two of the three nesting grounds for hawksbills in the highest range (>1,000 activities yr^{-1}) are located outside of the Eastern Caribbean, as are half of the sites in the next range (500-1,000 activities yr^{-1}). This makes Sandy Point even more important when analyzed in this regional context.

If activities of all species of turtles nesting at Sandy Point are included, the significance of this beach for sea turtle nesting is even greater. Nesting by leatherback sea turtles typically falls in the 500-1,000 activities yr^{-1} category and nesting by green turtles is in the largest category, with over 1,000 activities documented annually (Valiulis 2012, 2013). This makes Sandy Point extremely rare with such proportionally large nesting aggregations for multiple species on the same beach and it may be the only beach in East Caribbean that hosts large nesting populations of three different species. These results show that Sandy Point is an important nesting ground for hawksbill sea turtles in the U.S. Caribbean. It also confirms that the protection afforded to these beaches that are legally classified as a USFWS wildlife refuge is very important and has

prevented the uncontrolled development and disturbance of these nesting beaches. Survey efforts should be continued to confirm the regional significance of this nesting beach and to understand the population dynamics of turtles nesting at this site.

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CHAPTER 2

INTERNESTING DIVING BEHAVIOR OF HAWKSBILL SEA TURTLES

Introduction

Understanding the behavior of endangered species can be immensely useful in the formulation of management plans (Wikelski and Cooke 2006). For air-breathing marine animals like sea turtles, diving behavior is often used to understand movement and behavior patterns in the context of management of the adjacent aquatic habitat where turtle nesting beaches are protected. For example, diving behavior has been used to examine aquatic internesting habitat use and make management recommendations to reduce interactions between sea turtles and local fisheries and industrial longline fisheries (Polovina et al. 2003, Hays et al. 2004). Characterizing depth usage has shown that turtles in some areas may be prone to capture by trawlers due to their use of benthic habitats (Fossette et al. 2008). Analysis of diving behavior has also played a role in quantifying the risk posed to turtles by boat strikes (Sobin and Tucker 2008).

One of the challenges in understanding diving behavior is that it can vary geographically and temporally. As a result, diving patterns of conspecific individuals can be different in different habitats (Houghton et al. 2002). For females during the

internesting interval, the habitat offshore from the nesting beach influences diving behavior (Gaos et al. 2012b, Walcott et al. 2013). For example, leatherback dive depths are constrained during the internesting interval by the depth of the continental shelf surrounding the nesting beach (Wallace et al. 2005). Also, the quality of foraging habitat near nesting grounds may influence whether or not females forage during the internesting interval (Hochscheid et al. 1999, Hays et al. 2002b). To fully understand internesting diving behavior for a particular species, it is necessary to examine internesting diving behavior across multiple nesting grounds in order to account for site-specific differences in behavior.

Among all sea turtle species, hawksbill sea turtles are the most constrained to the tropics and usually inhabit coral reef habitats (Meylan 1988, León and Bjorndal 2002). In many of their nesting grounds in the Caribbean, shallow coral reef habitat is located nearby, providing a residence area during the internesting period (Starbird 1993, Walcott et al. 2012). However, at Sandy Point National Wildlife Refuge, there is not an extensive reef close to the nesting beach and the reef that does exist is highly polluted and degraded, (Oliver et al. 2011) which makes it a suboptimal place for hawksbills to spend the internesting interval. As a result, these turtles are likely to move further away from the nesting beach to locate a suitable internesting residence area.

As these movements occur, hawksbills are likely to encounter extremely deep water because the continental shelf descends abruptly close to the nesting beach (Figure 2.1). Other studies have shown that hawksbill diving behavior during the internesting interval is constrained by the bathymetry surrounding the nesting beach.

For example, eastern Pacific hawksbills inhabiting shallow estuaries spend 89% of the time in waters less than 10 meters deep (Gaos et al. 2012b). On Millman Island, Australia, which is also surrounded by shallow water, internesting hawksbills rarely dive deeper than 5 meters (Bell and Parmenter 2008). Diving behavior in these studies is likely constrained by the depth of the seafloor, as foraging individuals in sites with deeper water have been shown to dive to much greater depths (Storch 2004, Blumenthal et al. 2009b). I hypothesize that turtles nesting on Sandy Point will engage in deeper diving due to the presence of deep water offshore from the nesting beach. These previous studies also found very little variation in depth use, likely because the water around the nesting beach is uniformly shallow at these sites. Around St. Croix, the width of the continental shelf is highly variable, ending close to the shore in some locations, while extending several kilometers in others (Hubbard et al. 1981). I also hypothesize that this variability in depth around the nesting beach will result in large degrees of individual variation in depth usage during the internesting interval.

In this study, time-depth recorders were used to record internesting diving behavior of hawksbill sea turtles nesting at Sandy Point National Wildlife Refuge. Specifically, I aimed to investigate whether the unique bathymetry of Sandy Point affects diving behavior of these internesting hawksbills. Diving behavior was characterized by examining dive durations, surface durations, and dive depth to see how patterns in these parameters varied between turtles nesting on Sandy Point and those nesting on other beaches. Studying these patterns of diving behavior can lend insight into the types of activities in which turtles are engaging and help to better understand

how internesting hawksbills behave during this crucial life history stage. Furthermore, understanding how site-specific environmental features influence this behavior has implications for management plans across broader spatial scales.

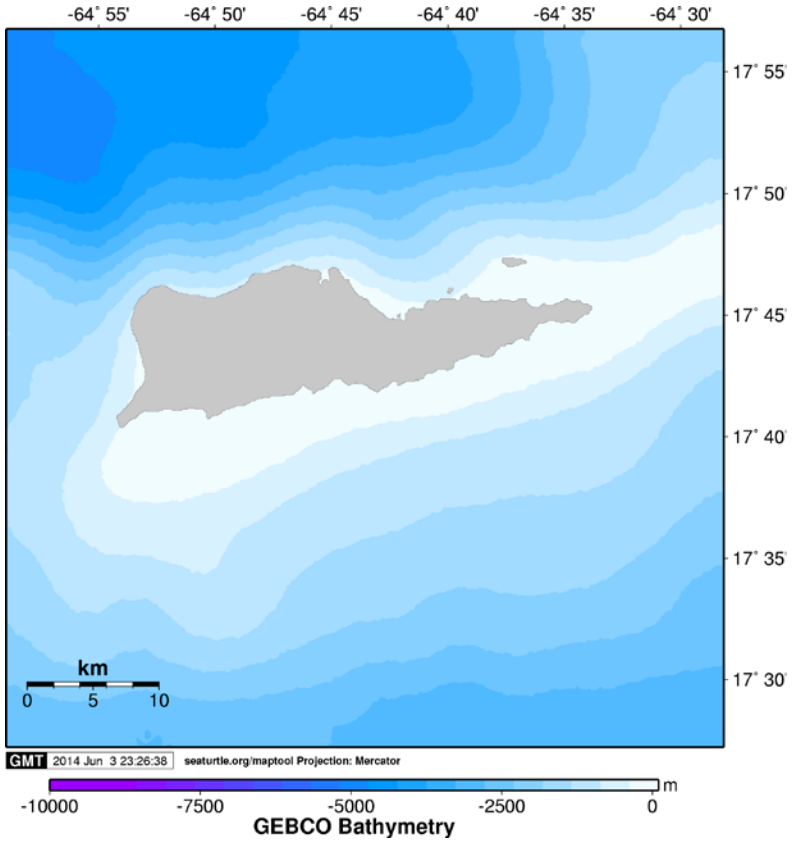

Figure 2.1: Bathymetry around St. Croix.

Methods

Diving behavior was studied using time-depth recorders (TDRs), model LAT 1100 manufactured by Lotek (Lotek Engineering, Newmarket, Ontario, Canada). These devices were set to measure pressure and temperature every 70 seconds and to

measure the wet/dry state every 5 minutes. These sample intervals were chosen to have the highest resolution while the TDR would still have the ability to record two internesting intervals if the turtle was not encountered on the nesting event subsequent to deployment but was encountered later in the season.

Turtles were tagged during oviposition and their reproductive status confirmed using ultrasonography (See Chapter 1). Because the device must be recovered to retrieve the data, TDRs were only deployed on turtles that would still lay more nests in the season, as determined by ultrasonography. When the turtle finished laying and started tamping, a 3mm hole was drilled on the left posterior marginal scute using a sterile drill bit. A sterile piece of surgical tubing with a diameter of 5mm was threaded through the hole and a segment of 400 pound fishing line inserted through the tubing. To anchor the TDR, the line was threaded through a plastic button on the ventral side of the carapace and then back up through the tubing. This left both ends of the line emerging through the tubing on the dorsal side of the carapace. The TDR had a hole on either end through which the line was inserted. A crimp was fastened to the lines to hold the TDR in place (Figure 2.2).

Figure 2.2: Placement of time depth recorder on supracaudal scute.

When a turtle with a TDR was encountered nesting again, the device was removed by cutting the fishing line with wire cutters. Pliers were used to remove the surgical tubing from the hole in the carapace. The device was removed as soon as the turtle was encountered to ensure successful recovery. After all the data were downloaded from the tags, they were reset and deployed on another turtle.

A dBASE program (dataBased Intelligence, Inc., Vestal, NY) was used to divide the resulting internesting data into diving and surfacing events. The surface was defined as the top 1.5m of the water column, so dives were only registered when the turtle descended below this threshold. This surface was defined to account for cases in which the TDR may not reach the water surface when the turtle surfaced to breathe and to account for waves rolling over the carapace while at the surface. Because turtles in this study had an average carapace length of 87.8 ± 3.9cm, a surface layer of 1.5m was

deemed to be appropriate to account for turtle surfacing behavior when the TDR failed to breach the surface of the water.

For each dive, the program calculated the dive duration, surface duration, and maximum dive depth. To examine changes in activity levels, I calculated the average dives per hour for each turtle for each quarter of the internesting interval. Because the data were not normally distributed, a Kruskal-Wallis test with post-hoc analysis was performed to determine pairwise comparisons in dives per hour between each quarter of the internesting interval. Each internesting interval was divided into day (6:00-18:00) and night (18:00-6:00) periods to examine diel patterns in diving. Diel dive and surface durations for each turtle were compared using a Mann-Whitney U Test. To examine diel patterns in activity levels, I used a nonparametric Levene's Test to compare equality of variances in depth measurements between day and night periods, with a greater variance indicative of more movement in the water column and thus higher activity levels. Depth data were binned into depth ranges for each turtle to examine the depths used across the course of the internesting interval.

Results

In 2012, two TDRs were recovered out of six deployments and in 2013, three TDRs were recovered from eight deployments. Two of the TDRs from 2013 were recovered after two internesting intervals had elapsed. Thus for both years there was a total of seven sets of internesting data obtained from five different individuals. A total

of 5403 dives were recorded over the course of 2506 hours of data collection. Deployment and recovery information is summarized in Table 2.1.

Table 2.1: Time depth recorder deployment and recovery data.

	Carapace length (cm)	Deployment Date	Retrieval Date	Days of Data
Turtle A	84.9	13/07/2012	27/07/2012	14
Turtle B	83.0	31/07/2012	13/08/2012	14
Turtle C	86.5	06/07/2013	07/08/2013	33
Turtle D	93.6	23/07/2013	24/08/2013	33
Turtle E	91.0	06/09/2013	20/09/2013	14

Analysis of dives per hour indicated Periods 1 and 4 were not statistically different ($p<0.05$), nor were periods 2 and 3 ($p<0.05$), but each one of these pairs was different from the members of the other pairs ($p>0.05$), with higher activity levels in periods 1 and 4 (Figure 2.3).

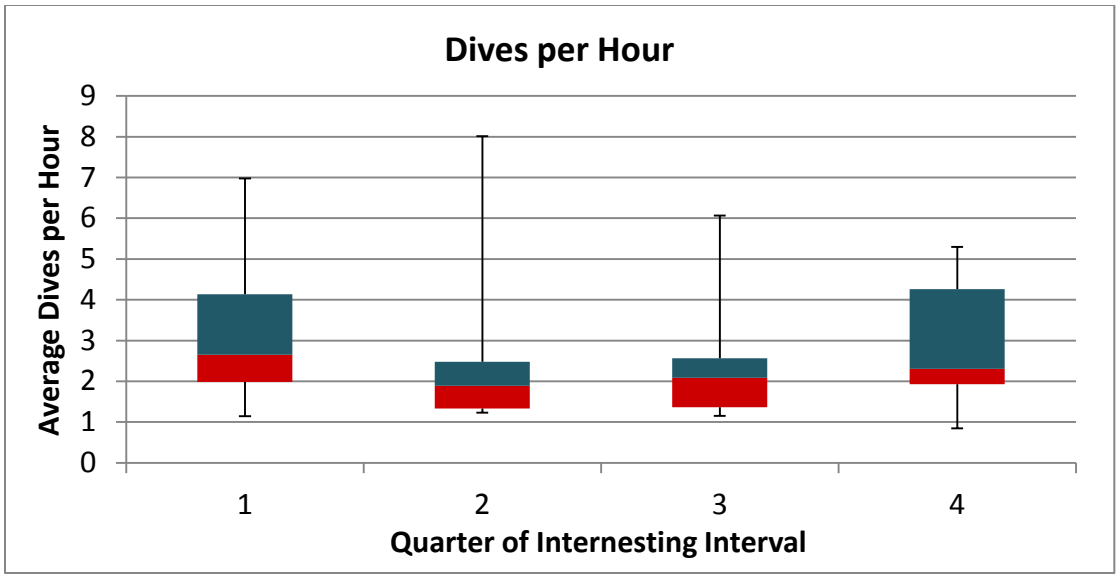

Figure 2.3: Box plots for dives per hour for turtles across each quarter of the internesting interval. Error bars indicate minimum and maximum values.

Turtles spent on average 86% of the internesting interval submerged, with average dive durations of 24.0 ± 22.3 minutes (range 1.2-157 min) and average surface intervals of 3.8 ± 13.9 min (range 1.2-422 min). Very little time was spent at the surface, with over 90% of all surface intervals being less than 5 min (Figure 2.4). With one exception, turtles spent over 60% of the internesting interval at the same depth range, but this depth varied between individuals (Figure 2.5).

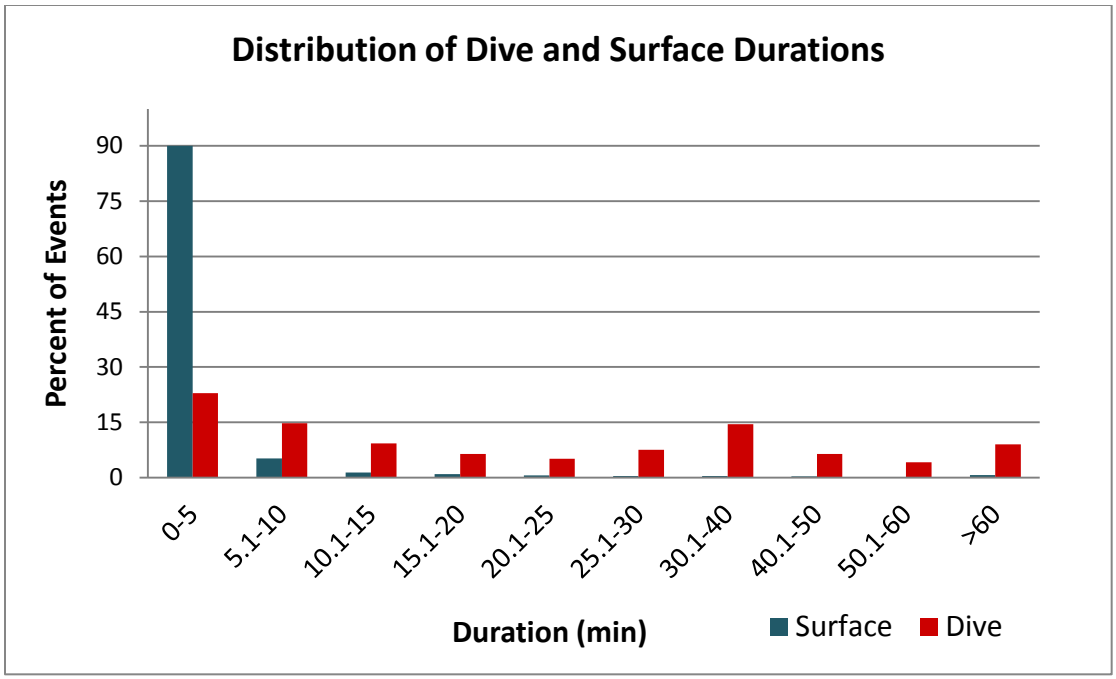

Figure 2.4: Distribution of dive and surface durations.

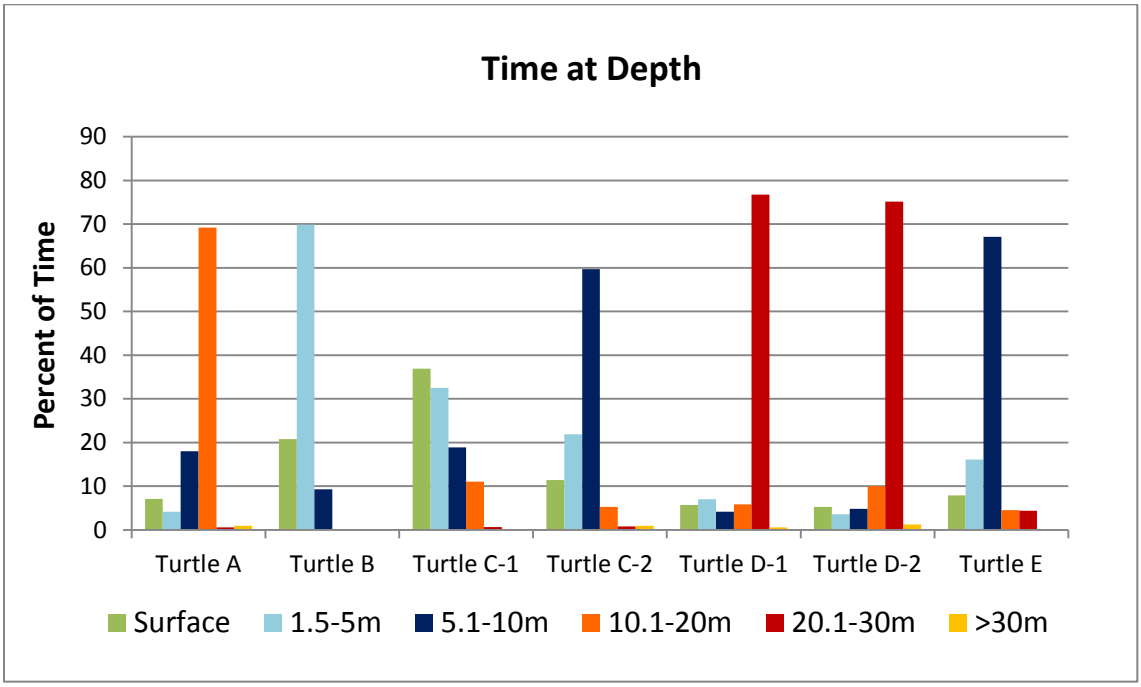

Figure 2.5: Time spent in each depth range.

Maximum dive depth for each turtle ranged from 10.8-94.6m. During the middle half of internesting, maximum dive depth remained relatively constant for each individual, with average interquartile ranges of 1.8 meters (Figure 2.6). Maximum dive depth increased markedly as the internesting interval progressed, with each turtle obtaining their deepest dives in the last 15% of the internesting interval (Figure 2.7). On average, the maximum dive depth attained in the last 15% of the internesting interval was 124% greater than maximum dive depth attained prior to that period. Dive parameters for each individual are summarized in Table 2.2.

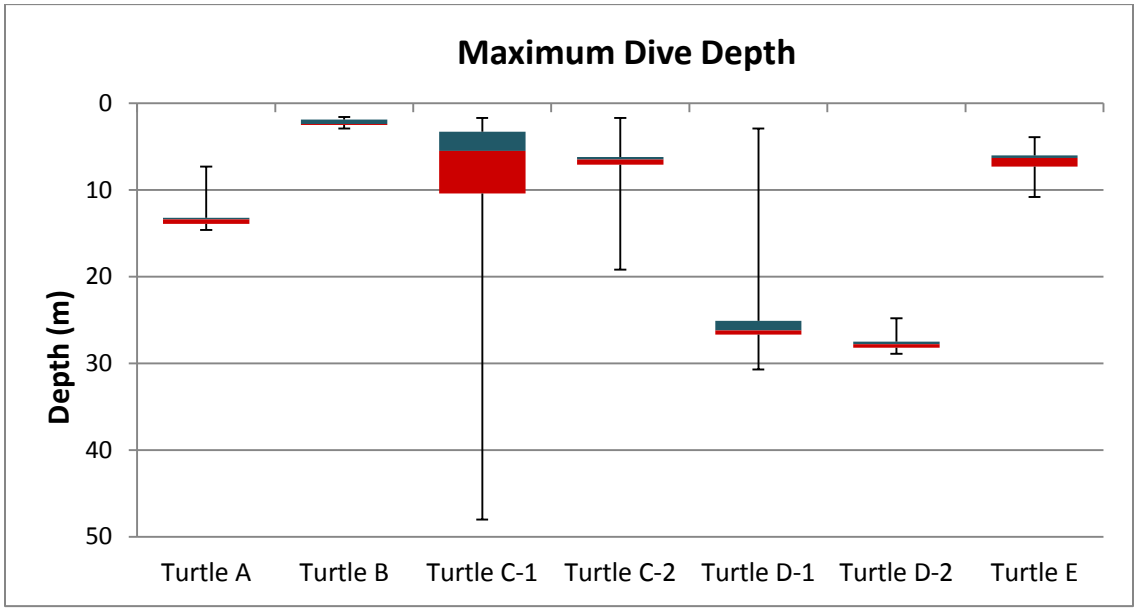

Figure 2.6: Box plots for maximum dive depth during the middle half of the internesting interval.

For most individuals, there was a greater variance in depth measurements at night and significantly longer dive durations during the day, which indicates greater activity at night. Turtle E was an exception to this, with greater variance in depth during the day and longer dive durations at night. There was no clear pattern in diel depth use or surface durations for these turtles, with both of these variables for all individuals split between being longer in day and night.

Table 2.2: Dive parameters for each turtle. 1 and 2 indicate the 1st and 2nd internesting interval recorded for those turtles.

Turtle	Percent Time Underwater	Avg Dive Duration (min)	Avg Surface Duration (min)	Max Dive Duration (min)	Max Surface Duration (min)	Max Depth (m)
A	92.9	32.2	2.5	90	61.2	84.4
B	79.2	19.4	5.1	134.4	427.2	10.8
C-1	63.1	10.3	6	70.7	386.7	47.9
C-2	88.6	18.2	2.4	157.3	46.7	46.3
D-1	94.8	50.2	2.8	93.3	104	94.6
D-2	94.3	45.3	2.7	96	42	32.5
E	92.1	28.9	2.5	130.7	81.3	27.9

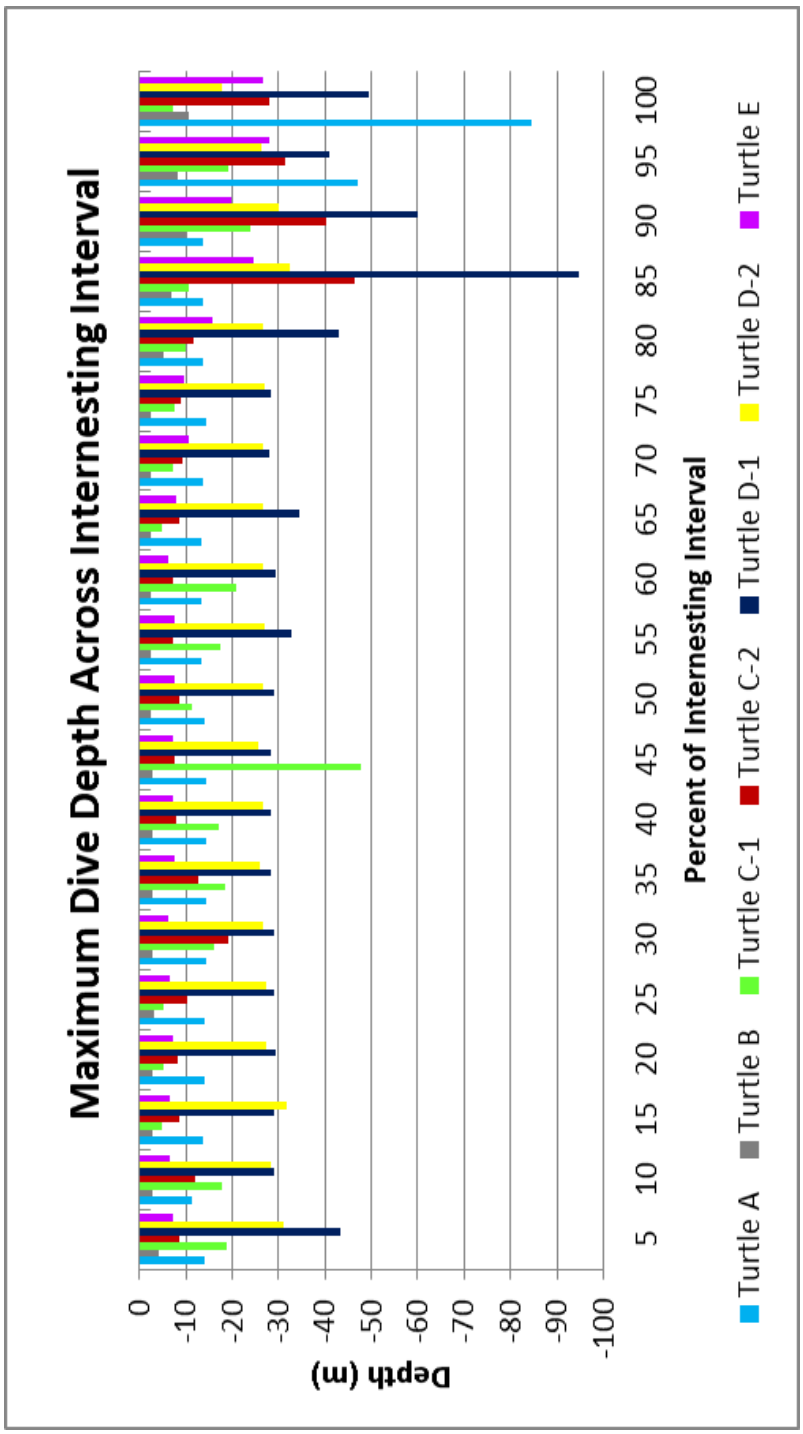

Figure 2.7: Changes in maximum dive depth across the internesting interval.

Temperatures recorded by the TDRs averaged 29.06 ± 0.45˚C (range 24.76-35.62; Figure 2.8). The highest and lowest temperatures occurred when turtles were at the surface. Excluding these temperatures experienced at the surface, water temperatures averaged 29.05 ± 0.42 °C (range 26.46- 31.46; Figure 2.9).

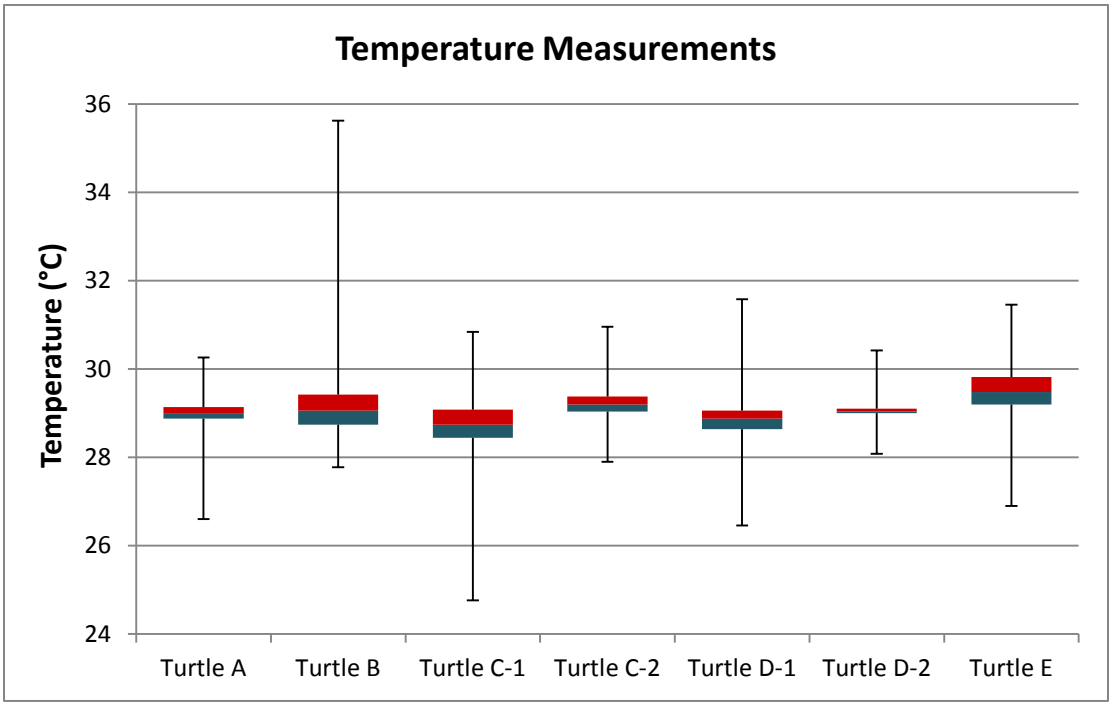

Figure 2.8: Box plots for temperature measurements. Error bars indicate minimum and maximum temperatures.

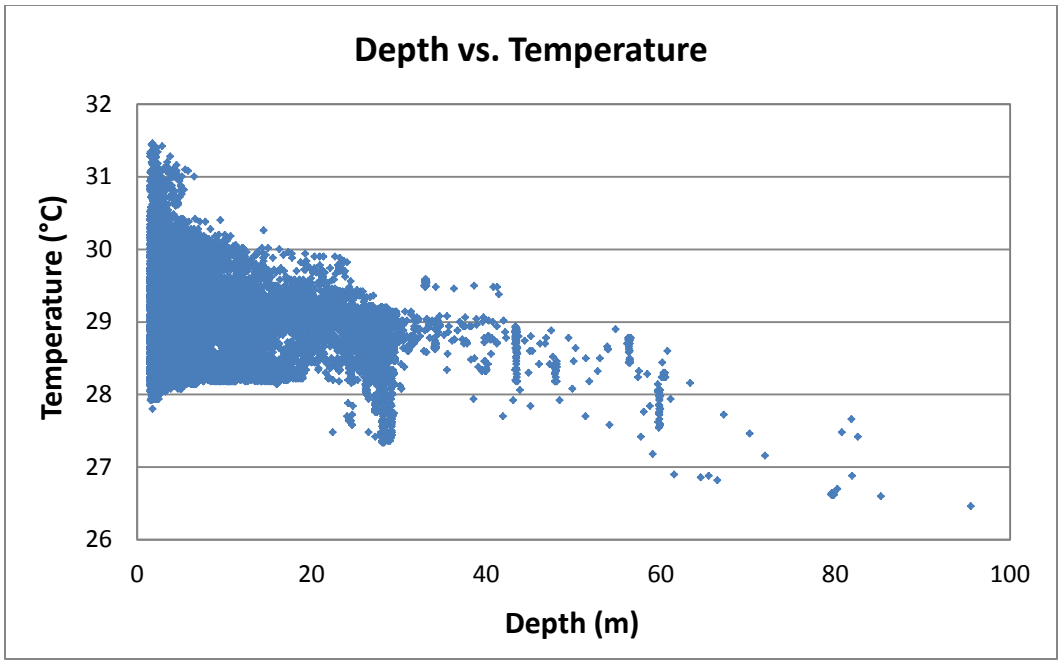

Figure 2.9: Changes in water temperature with depth.

Discussion

Measuring the dive depth of several turtles during their internesting interval provided insight into how these animals behaved and enabled investigation of whether environmental features around Sandy Point influenced diving behavior. Analysis of dives per hour indicated that these turtles were more active during the first quarter of the internesting interval, became less active during the middle half, and increased activity levels again in the last quarter. These activity patterns are similar to previously described movement patterns for hawksbills during the internesting interval (Walcott et al. 2012). As turtles move away from the nesting beach, they dive regularly, while throughout the middle half, they become significantly less active, suggesting they have taken up residence in a spatially restricted area. Activity levels pick up as turtles began

returning to the nesting beach to lay their next clutch (Storch 2004, Houghton et al. 2008, Walcott et al. 2012). The activity patterns I documented suggest that these turtles are likely engaging in similar behavior and thus do not differ in this respect from turtles on other beaches. The changes in activity levels can be seen clearly by comparing dive profiles spanning 12 hours from one individual (Turtle E) from the first (Figure 2.10), middle (Figure 2.11), and last (Figure 2.12) day of the internesting interval.

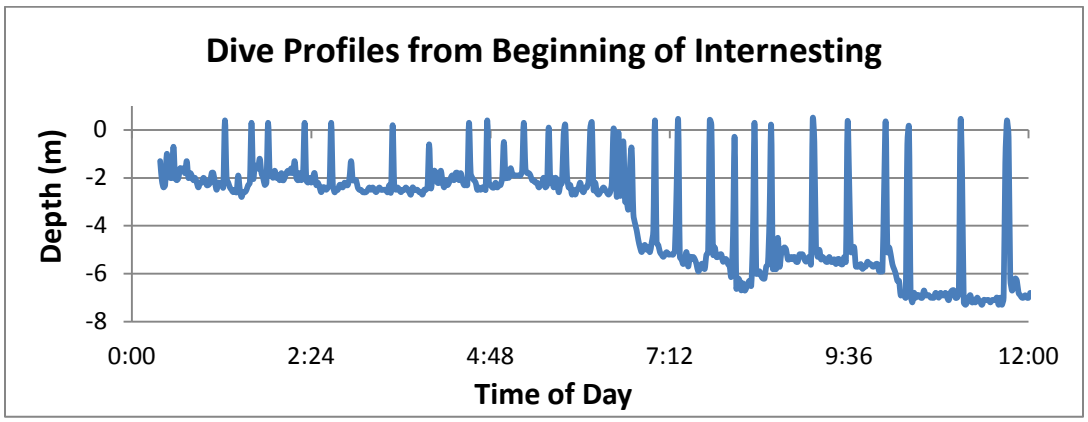

Figure 2.10: Dive profiles for Turtle E during the first 12 hours of the internesting interval.

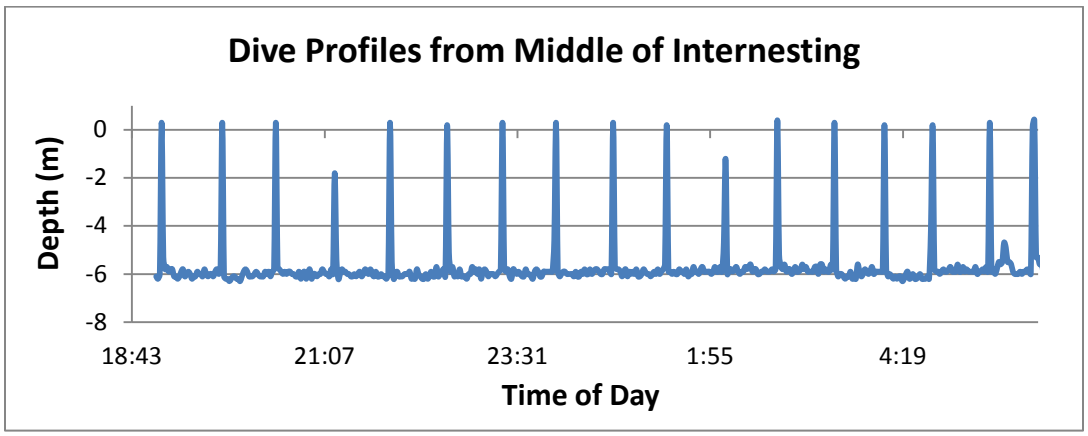

Figure 2.11: Dive profiles for Turtle E during day seven of the internesting interval.

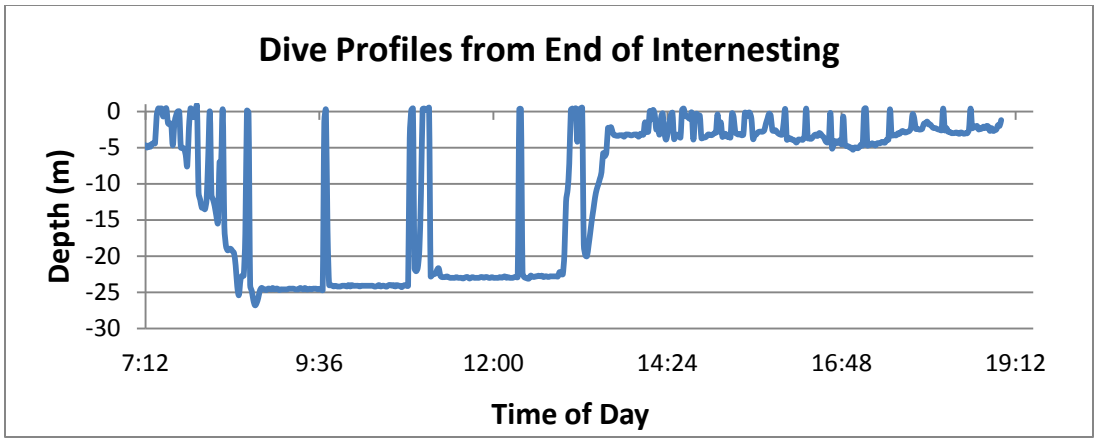

Figure 2.12: Dive profiles for Turtle E during the last 12 hours of the internesting interval.

Overall, I generally documented long dives to a constant depth and short surface intervals (see Figure 2.11), which is consistent with seafloor resting (Van Dam and Diez 1997). Dive durations averaged 29.1 min for all individuals, but similar to Gaos et al. (2012b) I also recorded some dives longer than 2 hours (n=8). As longer dive durations are linked to decreases in activity level (Hays et al. 1999, Okuyama et al. 2012), the turtles in this study are likely inactive for long periods of time, supporting the idea of seafloor resting. This is a widespread behavior reported for internesting hawksbills in the Caribbean (Storch 2004, Walcott et al. 2013) as well as in other oceans (Bell and Parmenter 2008, Houghton et al. 2008, Gaos et al. 2012b).

Resting on the bottom or in benthic reef habitats likely serves as a mechanism to conserve energy (Walcott et al. 2013), as reproductive migrations are energetically costly (Rivalan et al. 2005) and hawksbills are not thought to forage during the internesting interval (Santos et al. 2010). By remaining inactive during the internesting

interval, gravid females are able to diminish unnecessary energy expenditures and rely on fat stores acquired prior to migrating to the nesting ground (Kwan 1994, Hays et al. 2002a). Gravid females may even be able to significantly increase reproductive output during the nesting season by remaining inactive during the internesting intervals (Hays et al. 2000), so bottom or benthic resting behavior likely confers numerous advantages during this life history stage.

This conclusion of sedentary behavior is reinforced by analysis of depth usage. With one exception, each turtle spent the majority of time (over 60%) at the same depth range. This observation reiterates the lack of activity, as turtles would exhibit a more equal distribution of times across each depth range if they were diving often. The constant depth across two internesting intervals for Turtle D suggests fidelity to an internesting residence area, as has previously been reported for the species (Starbird 1993, Walcott et al. 2012). There was a noticeable amount of individual variation in the depths that these turtles inhabited, ranging from less than 5 meters up to 30 meters. This finding supports the hypothesis that turtles nesting on Sandy Point would show individual variation in depth utilization. Such variation is likely a result of the varying width of the continental shelf around St. Croix. In some locations the continental shelf extends several kilometers, whereas the narrow continental shelf in other areas results in steep cliff walls and underwater canyons located close to shore (Hubbard et al. 1981). These geological formations offer a wide variety of depths that turtles can inhabit, leading to profound individual variation in depth use during the internesting interval.

This is a contrast to hawksbills that inhabit inshore estuaries and mangrove forests, where there is not a wide range of depths available for use and dive depths are more uniform (Gaos et al. 2012a, Gaos et al. 2012b). The results of this study are similar to other hawksbills in the Caribbean. Immature hawksbills inhabiting a reef wall habitat in the Cayman Islands, were shown to utilize different depths, effectively partitioning habitat in a vertical dimension (Blumenthal et al. 2009b). Internesting hawksbills in Barbados were also shown to inhabit a range of depths, consistent with the variety of habitats present in the internesting areas (Walcott et al. 2013). Variation in depth usage for internesting hawksbills is likely a product of what types and depths of habitats are available to them during the internesting interval and may vary substantially across different geographical regions.

Despite the general trend of long dive durations, short surface durations, and relative inactivity, there were noticeable exceptions to these. For example, some cases of extremely long surface durations (>7 hours) were documented. However, these long surface durations were limited to two individuals. During the first internesting interval, Turtle C exhibited 12 surface intervals that were greater than 90 min and spent the largest percentage of time at the surface (33%). These extended surfacing events were temporally constrained, with 11 of them occurring between days 5 and 10 during her internesting interval of 17 days (Figure 2.13). Extended surface times may serve as a mechanism to remove lactic acid from the bloodstream after long bouts of diving (Hochscheid et al. 2010), but this is not likely the explanation for these surface times, as they were neither preceded nor followed by dives of long duration. An additional

explanation may be that the turtle was attempting to raise body temperature, as basking in the sunlight can appreciably increase the temperature of the carapace (Standora et al. 1982). This also seems unlikely, given that these extended surface times were divided equally between night (n=7) and day (n=5) and that temperature during this time fluctuated very little (average temperature 28.7 ± 4.2°C).

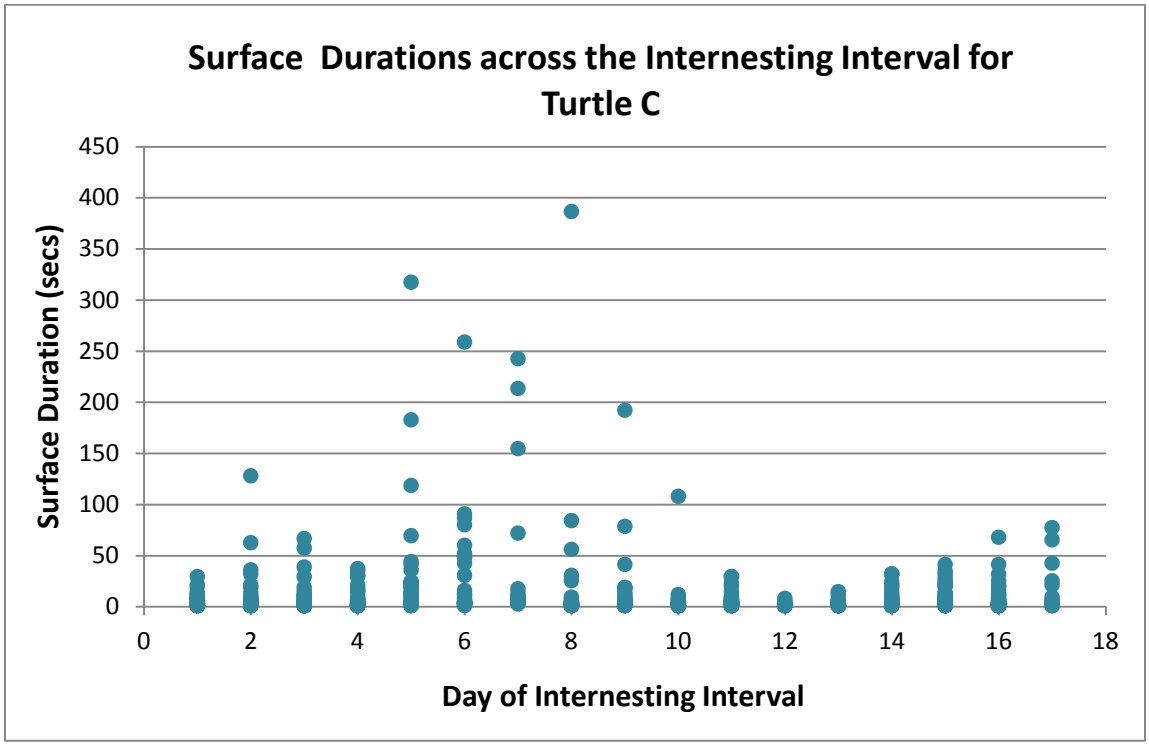

Figure 2.13: Length of surface durations across the internesting interval for Turtle C.

An alternative explanation may be that these surface intervals were cases of the turtle diving just below the surface of the water. The turtle may have been diving in the top 1.5 meters of the water column, which would have been registered as a surfacing event because of how we defined the surface. This would be consistent with other

studies, in which turtles engaged in shallow dives just beneath the surface, presumably to survey benthic habitats (Hochscheid et al. 1999, Walcott et al. 2013). Because this turtle did not have tags when encountered, it is possible that she was an inexperienced or first-time nester and that these surface intervals actually represent shallow dives in which the turtle was seeking out an appropriate residence area. This would be consistent with turtles in Barbados, in which the most movement during the internesting interval was observed in a neophyte, who may have been seeking out alternative residence areas (Walcott et al. 2012). This explanation seems likely, given that after day 10 of the internesting interval, there were no extended surface times. Additionally, this turtle was monitored over the course of two internesting intervals, and did not document any extended surface times during the second interval. In the second internesting interval, she dove repeatedly to the same depth that she did in the last 7 days of the first interval. This suggests that she located a suitable residence area in the last 7 days of the first internesting interval, then returned to it for the duration of the second internesting interval. As a result, extended periods of time at the surface ceased.

Turtles in this study tended to show greater activity levels at night, as indicated by greater variance in depth measurements and shorter dive durations. Trends in surface duration lacked consistency. Turtle E was an exception, and was clearly more active during the day, as can be seen by examining her dive profiles across a 24-hour period (Figure 2.14). Similarly, other studies on internesting hawksbills reported mixed results in diel behavior, with no strong diel influences on diving patterns (Bell and

Parmenter 2008, Gaos et al. 2012b). In contrast, studies on juvenile hawksbills have shown strong diel influences, with more diving occurring during the day (Van Dam and Diez 1996, Blumenthal et al. 2009b). However, these studies of juvenile hawksbills were conducted on foraging grounds, and diving occurred more during the day likely as a result of turtles searching for prey items. During the internesting interval, since turtles are not foraging, there may be less of an advantage to being more active during the day, which leads to the lack of strong diel influences on diving behavior.

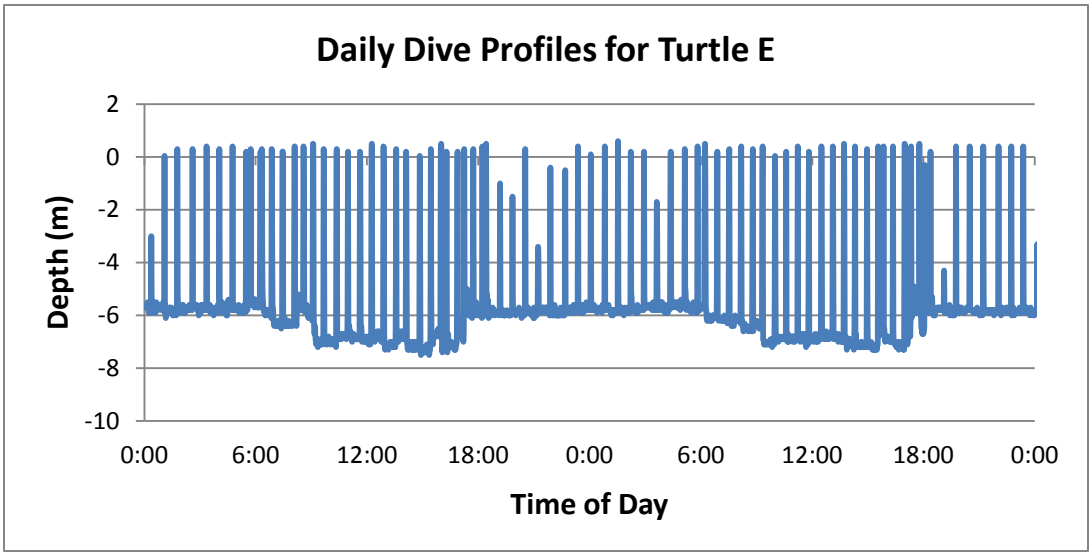

Figure 2.14: Dive profiles for Turtle E across 48 hours.

Where the results from this study diverged most significantly from previous studies on hawksbill internesting diving behavior was in maximum dive depths observed. Two of these turtles attained dive depths that exceed the deepest dive depth published for hawksbills during the internesting interval (84.4m and 94.6m). These

dives occurred in the last 15% of the internesting intervals. Although the other individuals did not dive as deep, they all attained their maximum dive depth in the last 15% of the internesting interval as well. During this time, the turtles were likely offshore from the nesting beach, beyond the continental shelf, as they waited to lay their next clutch.

In some of the deepest dives, turtles dove to the maximum depth and immediately ascended, while in others they remained at deep depths for extended periods. For example, Turtle A spent 30 minutes at 79 meters, 25 minutes at 40 meters, then stayed 58 minutes at 55 meters (Figure 2.15). Similarly, Turtle D remained at 59 meters for 43 minutes (Figure 2.16) and 47.5 meters for 44 minutes (Figure 2.17). These dives indicate seafloor resting, as turtles had been doing for the bulk of the internesting interval. When looking at the whole internesting interval, these results suggest that turtles nesting on Sandy Point spend the majority of the internesting interval inactive on the seafloor, and as they migrate back to the nesting beach, the water becomes much deeper, which causes the turtles to dive deeper to locate the bottom. As a result, extraordinarily deep dives for the species were observed that have not been recorded at other locations.

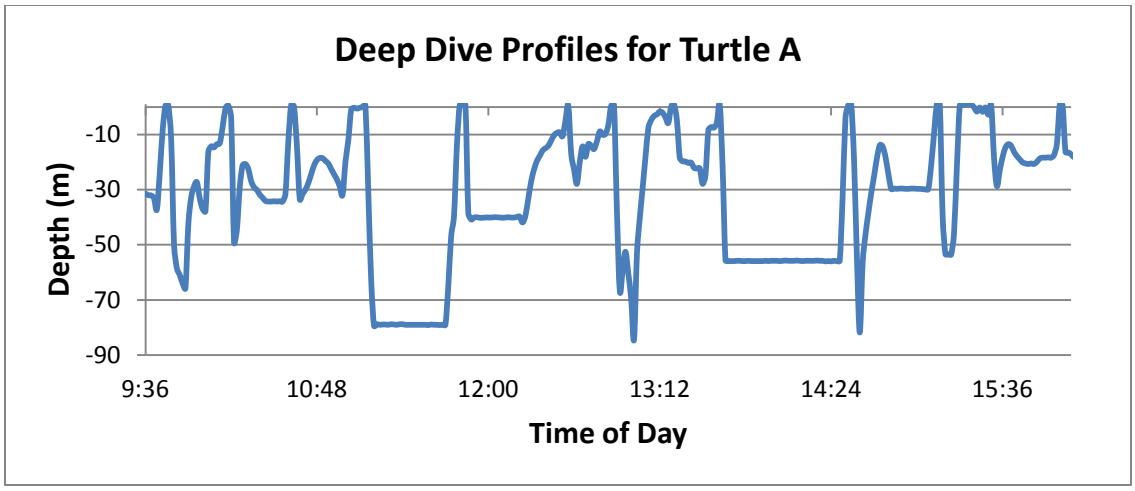

Figure 2.15: Deep profiles for Turtle A.

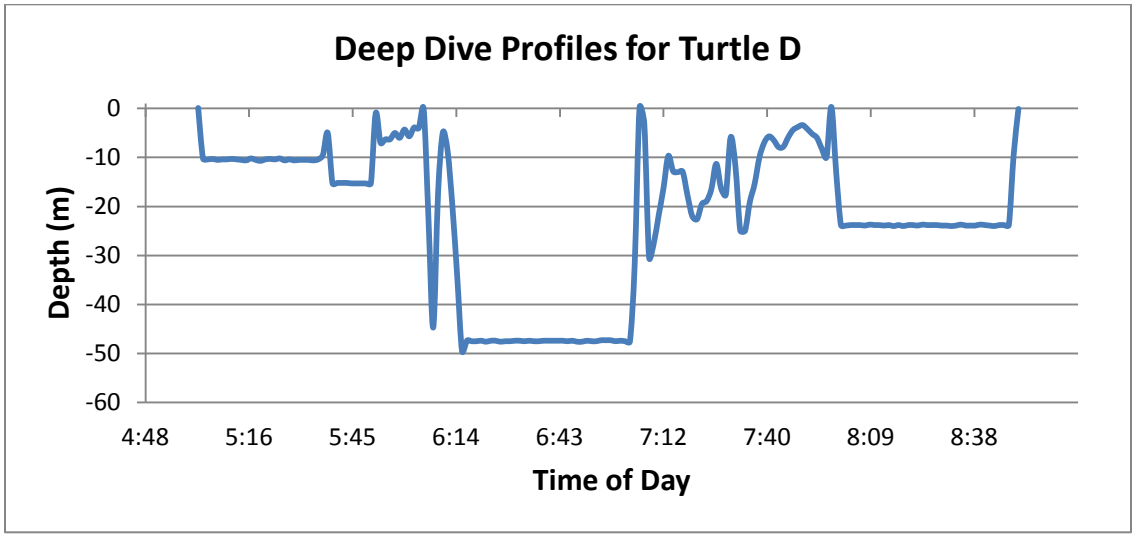

Figure 2.16: Deep dive profiles for Turtle D.

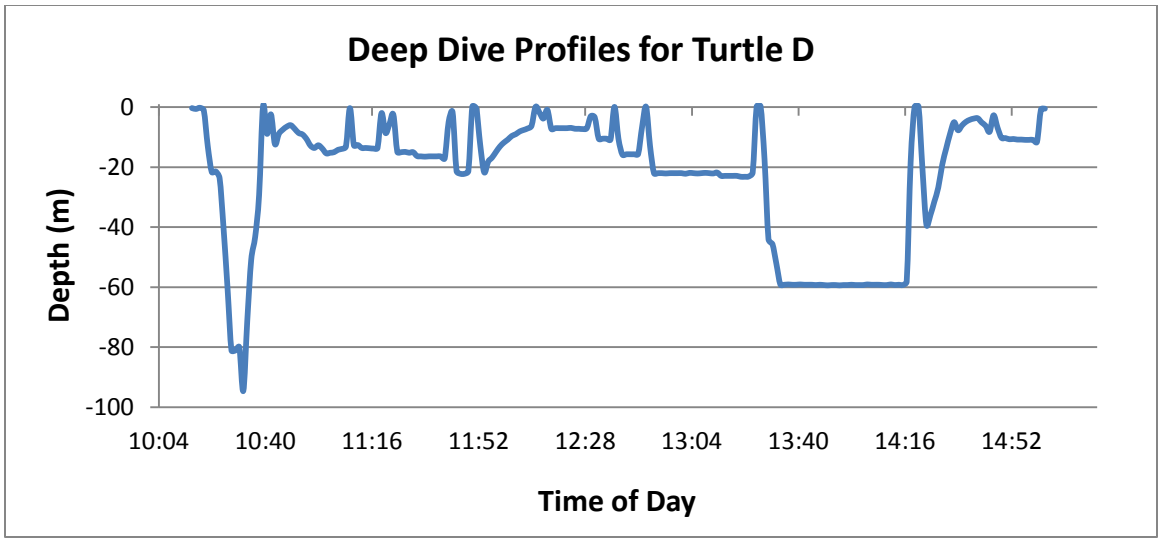

Figure 2.17: Deep dive profiles for Turtle D.

The reason that these turtles are physiologically capable of undertaking such deep dives is also influenced by the water temperature. Even when turtles were at depths greater than 80 meters, they never encountered water cooler than 26.5°C. 15°C is reported to be the lowest temperature at which turtles can maintain locomotion, and turtles can become comatose at temperatures lower than this, leading to cold-stunning and mortality (Davenport 1997). As a result, this 15°C thermocline is thought to play a role in regulating dive depth. Loggerhead turtles have been shown to change diving behavior in response to seasonal shifts in the thermocline, with the depth of dives corresponding to the depth of the thermocline (Howell et al. 2010). These seasonal shifts resulted in turtles staying above the 15°C thermocline 99% of the time even though dive depth changed seasonally (Howell et al. 2010). Similarly, internesting loggerheads in the Mediterranean have been shown to increase dive depths as the

nesting season progresses and water temperatures become warmer (Backof 2013). In this study, early in the season when the 15°C thermocline was located around 22 meters, they were constrained to the upper water column and rarely dove deeper than 2 meters. As the thermocline dropped to deeper than 50 meters over the course of the nesting season, dives became significantly deeper. Because the water never gets colder than 26.5°C in the top 100 meters in my study site, turtles were not constrained by the presence of cold water and therefore could engage in long bouts of deep diving that may not be possible at other locations.

An advantage to diving to such deep depths may be the avoidance of other turtles or predators such as sharks. Diving to the seafloor may serve to decrease intraspecific competition for resting areas, as nesting females have been shown to spar with one another in competition over resting sites (Schofield et al. 2007), which would be undesirable at a time when they are attempting to conserve energy. Spending time at deep depths may also be a way of avoiding males, as breeding male hawksbills can be found in close proximity to the nesting beach (Van Dam et al. 2008). Gravid leatherbacks have been shown to avoid interactions with males, increasing dive durations and sometimes remaining motionless on the seafloor in the presence of males (Reina et al. 2005). These turtles may also be avoiding other species of turtle, as there are large numbers of green turtles nesting on Sandy Point (over 1,000 nests were laid in 2012) and green and hawksbill nesting seasons overlap (Valiulis 2012, 2013). Additionally, there are breeding green males in the area, as evidenced by mating pairs washing ashore (J. Hill, pers. obs.). There is a lot of green turtle activity in the waters

around Sandy Point, and diving to very deep depths to locate a resting place may be advantageous if it allows gravid females to avoid interactions with other turtles, which have the potential to be energetically costly.

Spending time at deep depths may also serve as a means of predator avoidance by minimizing surface time (Seminoff et al. 2006). This may be particularly relevant at Sandy Point because there is evidence of shark predation on turtles. Nesting green turtles are frequently seen at this site missing rear flippers (J. Hill, pers. obs.; Figure 2.18B) consistent with shark predation (Heithaus et al. 2002) and leatherbacks are regularly documented with fresh injuries that may be the result of shark attacks (Garner et al. 2006, Garner and Garner 2007). Many of the leatherback injuries are documented when the turtle has already been seen nesting in the season without the injuries, indicating that the injuries occurred during the internesting interval (Figure 2.18A; J. Hill, pers. obs.). Furthermore, a tiger shark was caught off Sandy Point in 2011 that was found to have the head of an adult leatherback in its stomach (Shea 2011) and tiger sharks in other locations have been shown to prey on hawksbills (Young 1992, Blumenthal et al. 2009a). Although no recent wounds were documented on nesting hawksbills, this could be because the smaller size of the species means that bites from sharks are likely to be fatal. Decreasing predation risk may be one of the benefits of remaining inactive on the seafloor and could be one of the factors that lead to turtles continuing to seek out the seafloor even in areas of extremely deep water.

(A) (B)

Figure 2.18: (A) Fresh injuries on a nesting leatherback and (B) nesting green turtle missing rear flipper.

Studying the diving behavior of these turtles allowed for predictions regarding threats to which they are exposed during the internesting interval. The risk posed to turtles by boat traffic is low throughout most of the internesting interval because periods of time spent at the surface are brief and most of their time is spent resting on the seafloor. However, the risk increases the night of nesting, as dives are generally shallower and occur more frequently, and more time is spent at the surface. Fortunately, there has been a decrease in nightly boat traffic around Sandy Point, due in part to the recent closure of the oil refinery on the south shore, that decreases the potential for turtle-boat interactions (Valiulis 2013). This is supported by a 10% reduction in the percentage of leatherback wounds attributed to boat strikes since the closure of the refinery (Valiulis 2013).

The extensive time spent sedentary on the sea floor may also diminish the risk of fisheries interactions, as extensive movement would increase the potential for

encountering fishing gear. Turtles that are sedentary can be prone to submergence in bottom trawls (Carr et al. 1980), but trawling is not a fishing practice used in St. Croix. The conch fishery is large on St. Croix, but because capture is done by hand, it is very selective and bycatch is virtually non-existent (Kojis and Quinn 2006). Similarly, lobster fishing is widespread, but capture is done by hand (Kojis and Quinn 2006), or with the use of traps (Kojis 2004), which typically results in little bycatch (Matthews and Donahue 1997, Matthews et al. 2005). The greatest potential for hazard is likely through gillnets, which are staked to the ocean floor, but gill and trammel nets were outlawed in the US Virgin Islands in 2006 (Niesten and Gjertsen 2010). However, illegal use of the nets is still an issue and juvenile hawksbills have drowned in illegally set gill nets near Sandy Point (Shea 2010).

An additional threat may be in the form of derelict fishing gear. There is a pier in Frederiksted, north of Sandy Point that is a popular fishing spot, and there have been multiple cases of turtles drowned in discarded lines beneath the pier (Shea 2010, Lohr 2013) (J. Hill, pers. obs.; Figure 2.19). If hawksbills are moving through this area as they leave or approach the nesting beach, they may be exposed to a high risk of entanglement. Tracking the movement patterns of hawksbills during the internesting interval will provide much-needed insight into the potential for interactions with the hazards associated with the pier. Location data would also allow for an examination on a finer scale the threats they face in their particular internesting residence areas and should be a priority in future investigations of hawksbills nesting on Sandy Point.

Figure 2.19: Subadult hawksbill drowned under the Frederiksted pier. Fishing line is looped around the right front flipper.

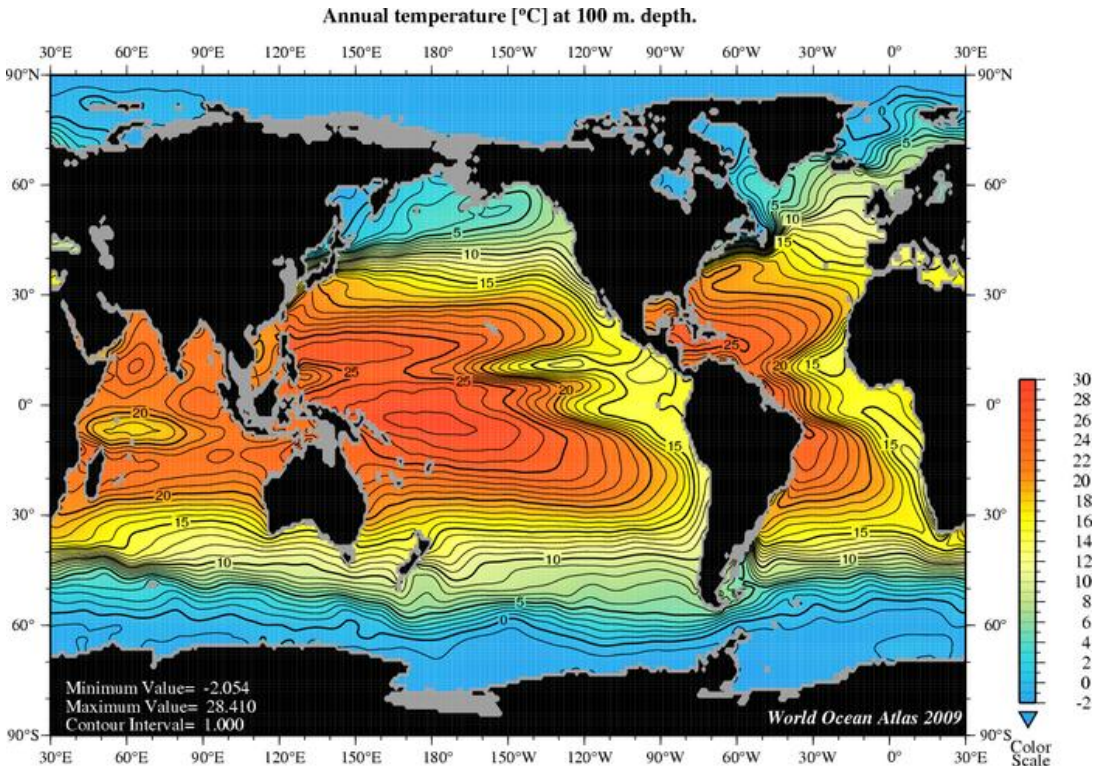

Figure 2.20: Global annual water temperatures at 100m depth. Provided by NOAA National Oceanographic Data Center.

Hawksbill sea turtles throughout their range have been shown to seek out the seafloor during the internesting interval (Storch 2004, Bell and Parmenter 2008, Houghton et al. 2008, Gaos et al. 2012b, Walcott et al. 2013). The results of this study confirm this behavior and show that when temperature is not a limiting factor, hawksbills will dive up to 100 meters deep to locate the seafloor. In the Indo-Pacific, hawksbills are likely to dive deep when bathymetry permits because the water temperature does not decrease much with depth (Figure 2.20). However, in the Eastern Pacific and Eastern Atlantic, temperature drops rapidly with depth, so hawksbills are likely to avoid cold waters by not diving to the seafloor. The findings of this study provide insight into hawksbill diving biology and can be applied to other sites to predict the diving behavior of hawksbills at particular locations, which is important in the formation of management plans for this critically endangered species.

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CHAPTER 3

POPULATION STRUCTURE OF NESTING HAWKSBILL SEA TURTLES ON ST. CROIX

Introduction

The life histories of hawksbill turtles are characterized by long periodic migrations and switching through different developmental habitats as they mature (Van Dam et al. 2008, Putman et al. 2014). Such complexity often makes it challenging to define populations, as their locations continually shift and sometimes mixed stocks of individuals that hatched from different beaches inhabit the same area (Bowen et al. 2007, Blumenthal et al. 2009). Molecular techniques provide valuable tools to resolve ambiguity regarding population structure, which can provide insight into hawksbill biology and behavior and can be used to inform effective management plans (Leroux et al. 2012). Genetic analysis has been particularly useful in determining the population structure on nesting grounds, which is used to define management units in order to appropriately direct conservation efforts (Wallace et al. 2010).

Hawksbill turtle nesting is spread throughout the Wider Caribbean, but there is little data on population structure for the majority of these rookeries. Genetic sampling across a large geographic range of rookeries is necessary for hawksbills because population structure tends to be complex, characterized by genetic connectivity

between distant rookeries, but genetic differentiation between proximate nesting grounds (Leroux et al. 2012). For example, hawksbills nesting on the leeward and windward sides of Barbados are genetically distinct, even though they are only separated by 30 kilometers (Browne et al. 2010). The nesting populations to which they are most genetically similar are thousands of kilometers away, with hawksbills from the leeward side of Barbados most similar to turtles nesting in Cuba and those from the windward side most similar to turtles nesting in U.S. Virgin Islands (Browne et al. 2010). Hawksbills nesting on different beaches in the Dominican Republic are also genetically distinct from one another (Carreras et al. 2013). These studies have shown that among all sea turtle species, hawksbills have the greatest genetic differentiation across small spatial scales (Browne et al. 2010). They also demonstrated the need for sampling across many nesting grounds in the Caribbean to gain further insight into this complex population structuring.

The island of St. Croix, part of the US Virgin Islands (Figure 3.1), contains many nesting beaches for hawksbill turtles (Mackay 2005), but the stock structure of nesting females from these beaches has not been thoroughly investigated. Buck Island Reef National Monument (17.789 N, -64.621 W) is located to the north of St. Croix and is the site of a long term monitoring project for hawksbill sea turtles. Stock structure has been documented for these turtles and in many studies used as representative of hawksbills nesting in the U.S. Virgin Islands (Bowen et al. 2007, Blumenthal et al. 2009, Leroux et al. 2012). Sandy Point National Wildlife Refuge is located on the southwest corner of St. Croix and is a large nesting ground for hawksbills (Chapter 1), but currently no data exist

on stock structure of hawksbills at this site. Over the course of three seasons of tagging nesting hawksbills on Sandy Point, 78 individual turtles were tagged and identified, none of which had been previously tagged on other beaches (Chapter 1). Tag returns have indicated that individual hawksbills do not migrate between Sandy Point and Buck Island for nesting (I. Lundgren, pers. comm.), even though they are located 40 kilometers apart by water. This data suggests that these two beaches may host separate stocks of nesting hawksbill sea turtles.

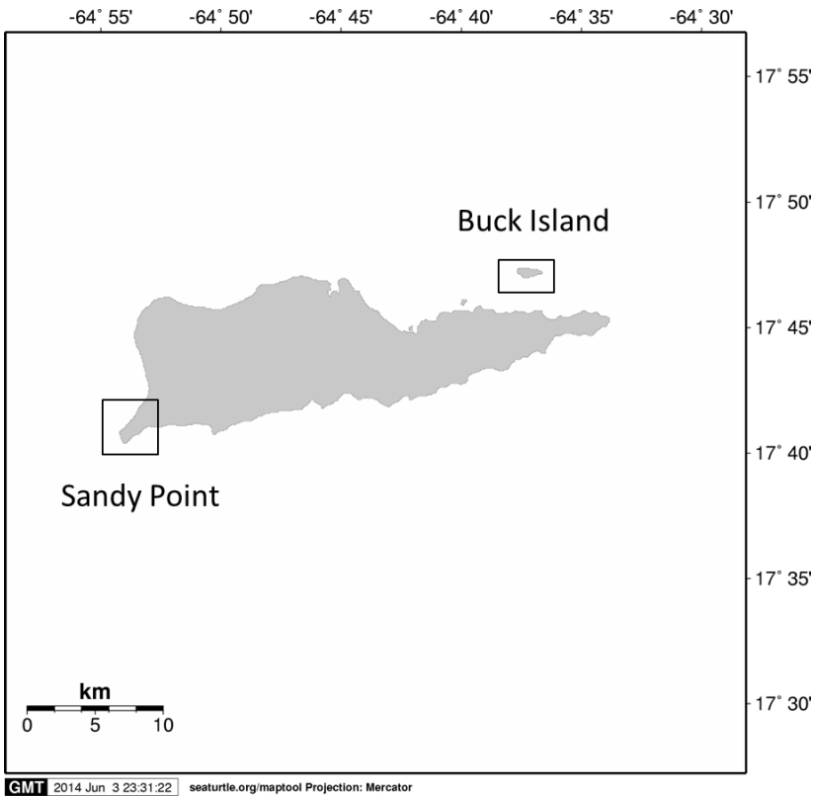

Figure 3.1: Map of St. Croix highlighting the locations of Sandy Point National Wildlife Refuge and Buck Island Reef National Monument.

In this study, mitochondrial DNA analysis from tissue biopsies was used to determine stock structure of hawksbills nesting on Sandy Point. This molecular technique has been used for other hawksbill nesting grounds, which allowed for comparisons between Sandy Point and other rookeries. The specific objective was to determine if these turtles are genetically distinct from those nesting on Buck Island and to ascertain the nesting beaches to which these turtles exhibited the greatest genetic similarity. This data was analyzed in the context of developing a management plan for hawksbills nesting at Sandy Point. Determining population structure of these turtles is considered a research priority in the Recovery Plan for Hawksbill Sea Turtles in the U.S. Caribbean (NMFS 1993).

Methods

Tissue samples were collected using an 8.0 mm biopsy punch from the trailing edge of the rear flipper of nesting hawksbills during oviposition (Hillis-Starr and Phillips 2002). I collected samples from 41 unique individuals. Samples were stored in 1.5mL vials in 96% ethanol at -20°C.

DNA was extracted from the samples using a Qiagen DNEasy extraction kit. I used the primers LCM-15382 (5' GCT TAA CCC TAA AGC ATT GG 3') and H950g (5' GTC TCG GAT TTA GGG GTT TG 3') to amplify 832 base pairs of the control region of the mitochondrial genome (Abreu-Grobois et al. 2006). The 25 µL polymerase chain reactions (PCR) consisted of water, 1 x Thermopol buffer, 10mM dNTPs, 10µM of each primer, 0.25 units of Taq polymerase and genomic DNA. The thermal cycling profile

consisted of the following: an initial DNA denaturation for 2 minutes at 90°C, followed by 30 cycles of (1) DNA denaturing at 94°C for 50 seconds, (2) primer annealing at 56°C for 50 seconds, and (3) primer extension at 72°C for 1 minutes, followed by a final primer extension at 72°C for 5 minutes.

PCR products were confirmed using gel electrophoresis in a 2% agarose gel stained with ethidium bromide. In cases where PCR products did not show up on the gel, PCR was performed again, but with the following changes: Taq polymerase doubled to 0.5 units, genomic DNA was increased, and addition of 1 µL of bovine serum albumin.

The PCR products were purified by combining 5µL of PCR product with 2µL of a combined Exonuclease I and Shrimp Alkaline Phosphate solution (ExoSAP-IT®) and incubating for 15 minutes at 37°C, followed by 15 minutes incubation at 80°C. Both forward and reverse strands were sequenced using an ABI® Big Dye Terminator v3.1 Cycle Sequencing Kit and analyzed with Applied Biosystems® model 3730 automated genetic analyzer. The 12 µL cycle sequencing reactions consisted of 1µM primer, 1:1 Big Dye/Buffer, and 3 µL PCR product. Cycle sequencing was performed under the following conditions: an initial DNA denaturation at 96°C for 1 minute, followed by 30 cycles of (1) DNA denaturation at 96°C for 10 seconds, (2) primer annealing at 50°C for 5 seconds, and (3) primer extension at 60°C for 4 minutes. Sequences were aligned, edited, and cropped using the program SeqScape v2.5 (Applied Biosystems®). Haplotypes were designated by comparing sequences to a reference library.

I used ARLEQUIN version 3.5 (Excoffier and Lischer 2010) to calculate haplotype (*h*) and nucleotide (π) diversity for these samples. To determine the amount of genetic

differentiation between Sandy Point and other rookeries in the Wider Caribbean, I used the haplotype rookery profiles presented in LeRoux et al. (2012) and Carreras et al. (2013) and used ARLEQUIN to calculate pairwise F_{st} comparisons (conventional haplotype frequency based), as well as exact tests of population differentiation using 10000 steps in the Markov chain and 10000 dememorization steps. Haplotype frequencies were also compared between Sandy Point and other rookeries using Chi-square tests with Monte Carlo resampling with 1,000 iterations, as implemented in the program CHIRXC (Zaykin and Pudovkin 1993). To graphically relate genetic distances between rookeries, I used pairwise F_{st} values to construct a neighbor-joining tree in MEGA version 4.1 (Tamura et al. 2007) and performed a principal coordinate analysis using GENALEX version 6.4 (Peakall and Smouse 2006).

Results

Haplotype diversity was 0.3305 ± 0.081 and nucleotide diversity was 0.013 ± 0.007. The haplotypes consisted of EiA01 (n=33), EiA11 (n=7), and EiA03 (n=1). Haplotype data for each turtle is presented in the Appendix. Every comparison (exact test, pairwise F_{st}, and chi-square) between Sandy Point and other rookeries were statistically significant, indicating genetic differentiation. Buck Island was very distantly related to Sandy Point, with an F_{st} value of 0.501 ($p<0.001$). The most similar rookery to Sandy Point was Brazil, ($F_{st}= 0.052$, $p=0.03$) followed by Cuba ($F_{st}= 0.0988$, $p< 0.001$), Barbados Leeward ($F_{st}= 0.052$, $p< 0.001$), and Antigua ($F_{st}= 0.052$, $p< 0.001$). Values for statistical analyses are summarized in Table 3.1.

Table 3.1: Values from statistical analyses of comparisons between Sandy Point and other rookeries in the Wider Caribbean. For exact tests, p values are shown. All Chi Square and F_{st} Values are significant, with p <0.001 in all cases. The only exception is F_{st} value for Brazil (p=0.03). ‡Data from LeRoux et al. (2012). *Data from Carreras et al. (2013).

	N	χ^2	F_{ST}	Exact Test
Buck Island‡	67	51.76	0.501	0.0001
Antigua‡	72	25.10	0.185	0.0001
Barbados Leeward‡	54	11.51	0.178	0.0005
Barbados Windward‡	30	38.21	0.504	0.0001
Brazil‡	66	21.58	0.052	0.0001
Costa Rica‡	60	76.13	0.440	0.0001
Cuba‡	70	14.66	0.099	0.0070
Guadeloupe‡	72	99.99	0.818	0.0001
Nicaragua‡	95	102.18	0.473	0.0001
Mexico‡	20	61.00	0.683	0.0001
Puerto Rico‡	109	104.00	0.441	0.0001
Dominican Republic-Jaragua*	16	40.34	0.430	0.0001
Dominican Republic-Saona Island*	33	41.37	0.480	0.0001

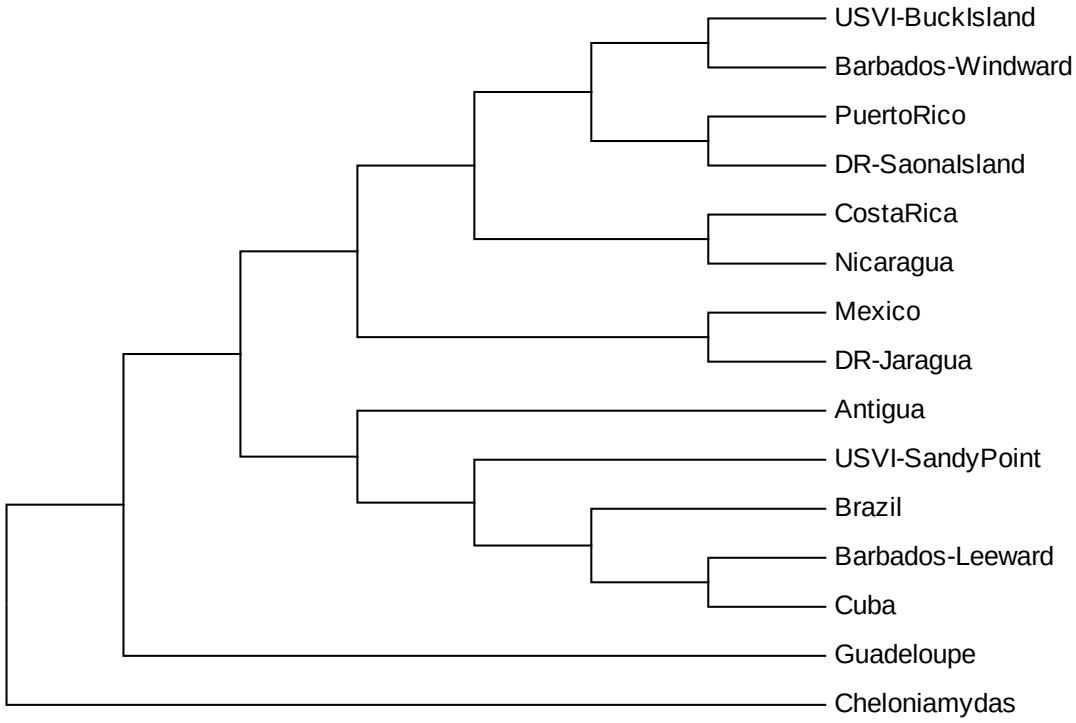

Figure 3.2: Neighbor-joining tree based on pairwise F_{st} values for hawksbill rookeries in the Wider Caribbean. *Chelonia mydas* (green sea turtle) is used as an outgroup.

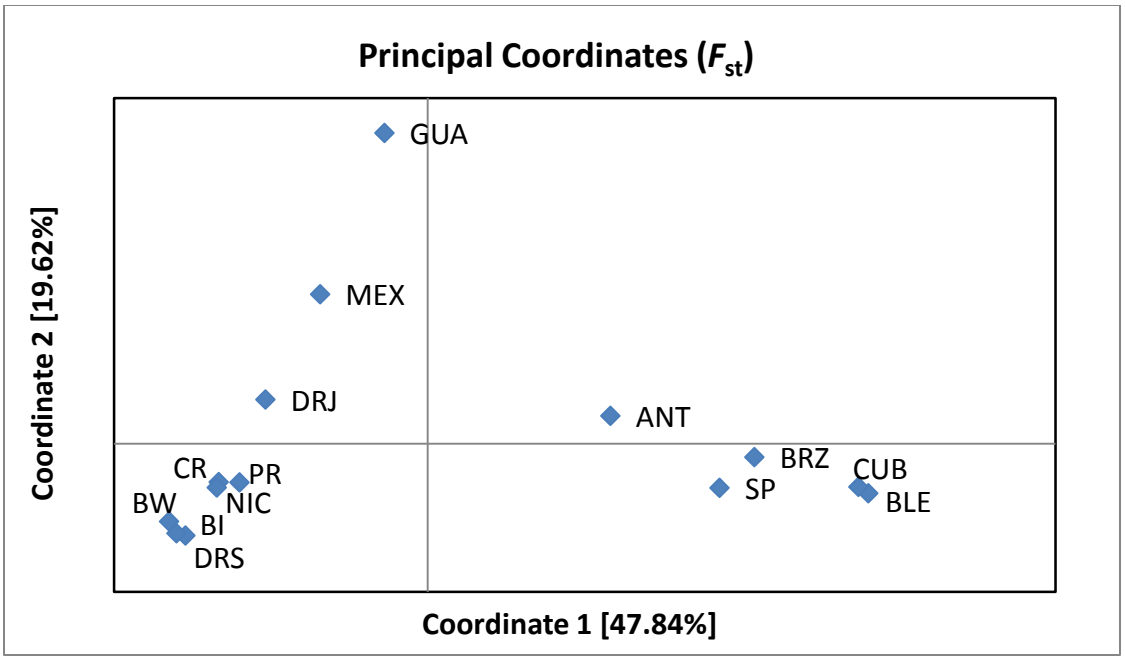

Figure 3.3: Principal coordinate analysis for pairwise genetic distances (F_{st}) between hawksbill rookeries in the Wider Caribbean. The percentage of variation explained by each principal coordinate in shown in brackets. SP=Sandy Point; ANT=Antigua; BLE=Barbados Leeward; BW= Barbados Windward; BI=Buck Island; BRZ=Brazil; CR=Costa Rica; CUB=Cuba; DRJ=Dominican Republic-Jaragua; DRS=Dominican Republic-Saona Island; GUA=Guadeloupe; MEX=Mexico; NIC=Nicaragua; PR=Puerto Rico.

Discussion

Hawksbill nesting stocks in the Wider Caribbean fall into one of the four following broad phylogenetic groups: 1) rookeries with a predominance of haplotype EiA01; 2) rookeries with a predominance of haplotype EiA11; 3) Guadeloupe, with a predominance of haplotype EiA9; and 4) Mexico, with a divergent haplotype profile (Leroux et al. 2012). Buck Island has a predominance of haplotype EiA11, making it a member of the second group, while EiA01 is the dominant haplotype at Sandy Point,

making it a member of the first group. This deep level of differentiation is reinforced by the significant values obtained through all statistical analyses ($p < 0.001$, all cases).

Additionally, despite the proximity of the two rookeries, they are more genetically similar to distant populations than to one another. Buck Island is genetically indistinguishable from the windward side of Barbados, while Sandy Point is most similar to Brazil. These relationships are apparent in the neighbor-joining tree (Figure 3.2) and principal coordinate analysis (Figure 3.3), which show the clustering of Sandy Point with the other members of group 1 (Antigua, Brazil, Cuba, and Barbados-Leeward), while Buck Island is clustered with rookeries belonging to group 2.

These findings further reinforce the patchy genetic relationships among hawksbill rookeries in the Wider Caribbean, which show differentiation among proximate rookeries, but similarity between rookeries that are separated by thousands of kilometers (Leroux et al. 2012). Hawksbills nesting at Sandy Point are genetically distinct from those at Buck Island which is located 40 kilometers away, but closely resemble a nesting population in Brazil that is over 2000 kilometers away. These results also reiterate the possibility for genetic differentiation across very small spatial scales, as previously reported for nesting beaches in the Dominican Republic (Carreras et al. 2013) and Barbados (Browne et al. 2010).

The reason for the connectivity between distant nesting grounds may be a result of ocean currents, which carry hatchlings away from the nesting beach. Blumenthal et al. (2009) used hatchling drift models to determine where ocean currents were likely to carry passively floating hatchlings and showed that particles from Barbados were carried

across the Caribbean Sea on the Caribbean Current, as were particles from Venezuela and Cuba. By contrast, many particles from Buck Island and Puerto Rico were carried northward and became entrenched in the Bahamanian ecoregion. Sandy Point faces south, and if hatchlings swim south when they reach the ocean, they are likely to encounter the Caribbean current. Entering the same current as hatchlings from the other rookeries defined by the predominance of haplotype EiA01 may provide connectivity and explain the genetic similarity between these distant nesting beaches. Ocean currents may therefore be a better predictor of genetic relationships between rookeries than geographic distance.

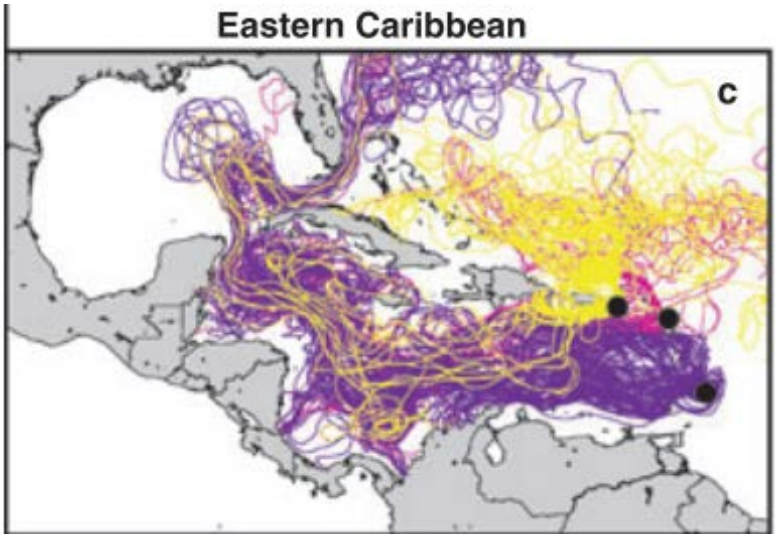

Figure 3.4: Hawksbill hatchling dispersal models. From Blumenthal et al. 2009.

This study supports the delineation of hawksbills nesting at Sandy Point and Buck Island into two separate entities for management purposes. As such, they should be

evaluated independently to assess threats and population status, and recovery plans should target the unique threats facing each rookery. For example, hawksbills nesting on Sandy Point are probably also nesting on nearby beaches on the south shore. Many of these are small and isolated and there are cases of eggs being poached on these beaches (C. Lombard, pers. comm.). The poaching of these nests may impact nesting populations on Sandy Point, but is not likely to impact Buck Island because they are a separate nesting population. Similarly, mongoose predation on hawksbill nests is prevalent on St. Croix, but eradication of mongoose at one of these sites is not likely to incur any benefit to nesting populations at the other site. Since nesting females do not go between beaches for nesting, the extirpation of hawksbills on Sandy Point would have long term impacts because turtles from Buck Island would not be expected to colonize Sandy Point in the near future (Bowen et al. 1993). Because these nesting sites host separate nesting stocks, conservation and management issues should be addressed independently on both beaches to ensure the viability of both populations.

This study illustrates the complexity of understanding and managing species that exhibit population partitioning across small spatial scales. The USVI consists of St. Croix, St. Thomas, and St. John, which together form one political entity. However, this political assemblage has no relevance in terms of hawksbill population ecology because there are multiple stocks of nesting hawksbill sea turtles within it. As a result, a single management plan is not likely to be adequate for all nesting beaches in the territory, and recovery efforts should be tailored to the threats facing each genetically distinct nesting population. Additionally, a single nesting beach from the USVI is not genetically

representative of all nesting beaches in the territory. Several studies of mixed stock analysis that were used to show the contribution of the USVI to foraging grounds have used Buck Island as a representative site for the territory (Bowen et al. 1996, Bowen et al. 2007). However these analyses likely do not fully assess the contribution of the USVI because only one of multiple stocks of nesting hawksbills was included. Future region-wide analyses should include samples from both Buck Island and Sandy Point to account for the genetic differentiation between these two nesting beaches.

Genetic analyses should be expanded to include nesting sites on the east end of St. Croix to gain a better understanding of hawksbill population partitioning around the island. Hawksbills at these sites have been shown to nest on both east end beaches and Buck Island (Mackay 2005), so they may both be part of the same genetic stock. Additionally, since green turtles nest at both Buck Island and Sandy Point, stock structure should be compared between these sites to determine if green turtles exhibit the same patterns we have documented for hawksbills. Of the 104 green turtles documented nesting in 2014, only 2 had been documented nesting on Buck Island, which suggests they may also be a separate stock (J. Hill, unpublished data).

This study provides further insight into hawksbill population structure by showing genetic differentiation in rookeries across very small spatial scales. The population partitioning I documented has important implications for developing management plans for this species. When multiple rookeries exist within the same political entity, the population structure among these beaches should be determined in order to ensure that recovery plans target the threats faced by each population. This

partitioning should also be taken into consideration when performing region-wide genetic analyses to fully understand population structuring across large geographic scales.

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APPENDIX

TURTLE TAG AND HAPLOTYPE DATA

APPENDIX

TURTLE TAG AND HAPLOTYPE DATA

	Pit Tag	Front Flipper Tag	Rear Flipper Tag	Haplotype	Years Encountered
1	NF	BBQ812	NF	EiA01	2013
2	151609316A	BBQ815	NF	EiA01	2013
3	NF	BBQ828	NF	EiA01	2012
4	NF	BBQ829	NF	EiA01	2012
5	151615764A	BBQ833	NF	EiA01	2013
6	NF	BBQ834	NF	EiA01	2012
7	NF	BBQ835	BBQ836	EiA01	2012
8	NF	BBQ838	NF	EiA01	2012
9	NF	BBQ839	NF	EiA01	2012
10	NF	BBQ841	NF	EiA01	2012
11	NF	BBQ843	NF	EiA01	2012
12	NF	BBQ844	NF	EiA01	2012
13	NF	BBQ845	NF	EiA01	2012
14	NF	BBQ848	NF	EiA01	2012
15	151617680A	BBQ849	BBQ850	EiA01	2013
16	151615576A	BBQ876	NF	EiA01	2013
17	151617096a	BBQ877	NF	EiA01	2013
18	151608401A	BBQ896	NF	EiA01	2013
19	NF	BBQ907	NF	EiA01	2013
20	151614224A	BBQ923	NF	EiA01	2013
21	151611377A	BBQ940	NF	EiA01	2013

22	151616434A	BBQ947	NF	EiA01	2013
23	NF	BBQ950	NF	EiA01	2013
24	132131310A	NF	NF	EiA01	2013
25	151603032A	TTH383	NF	EiA01	2013
26	132245130A	TTZ559	NF	EiA01	2011
27	151614080A	UUK862	UUK860	EiA01	2011,2013
28	151616311A	UUK961	UUK909	EiA01	2011,2013
29	151607041A	UUK976	NF	EiA01	2012
30	151602695A	UUK979	UUK870	EiA01	2011
31	NF	XXZ170	NF	EiA01	2012
32	NF	YYL732	NF	EiA01	2012
33	151610321A	BBQ832	NF	EiA03	2013
34	151606242A	BBQ807	BBQ808	EiA11	2011,2013
35	151613532A	BBQ811	NF	EiA11	2013
36	151612513A	BBQ830	NF	EiA11	2013
37	NF	BBQ837	NF	EiA11	2012
38	NF	BBQ842	NF	EiA11	2012
39	151615305A	BBQ856	NF	EiA11	2013
40	151606124A	UUK891	UUK892	EiA11	2011,2013
41	NF	BBQ840	NF	NS	2012
42	151610214A	BBQ801	TTH398	NS	2011
43	NF	BBQ817	TTH380	NS	2011
44	NF	BBQ819	NF	NS	2011
45	151618221A	BBQ822	BBQ821	NS	2011
46	151613765A	BBQ823	BBQ824	NS	2011
47	NF	BBQ853	NF	NS	2013
48	151613463A	NF	NF	NS	2011
49	NF	TTH301	NF	NS	2011
50	151607470A	TTH303	TTH304	NS	2011
51	151605460A	TTH307	TTH308	NS	2011
52	151611351A	TTH317	TTH315	NS	2011

53	NF	TTH319	NF	NS	2012
54	151616537A	TTH320	NF	NS	2011
55	151604432A	TTH323	TTH313	NS	2011
56	151605021A	TTH354	TTH353	NS	2011
57	NF	TTH361	NF	NS	2011
58	151607662A	TTH373	TTH336	NS	2011
59	151620416A	TTH376	TTH375	NS	2011
60	NF	TTH377	NF	NS	2011
61	151608663A	TTH382	TTH387	NS	2011
62	151616192A	TTH399	TTH400	NS	2011
63	151602672A	UUK863	UUK971	NS	2011
64	151605605A	UUK887	UUK888	NS	2011
65	NF	UUK890	UUK933	NS	2011
66	151618426A	UUK896	UUK944	NS	2011
67	151615014A	UUK915	TTH369	NS	2011
68	NC	UUK940	NF	NS	2011
69	151615433A	UUK942	UUK941	NS	2011
70	NF	UUK943	NF	NS	2011
71	151606734A	UUK951	TTH378	NS	2011
72	151603345A	UUK974	UUK973	NS	2011
73	NF	UUK977	NF	NS	2012
74	151615532A	UUK986	UUK985	NS	2011
75	151603573A	UUK993	UUK992	NS	2011
76	NF	UUK996	NF	NS	2012
77	NF	UUK997	NF	NS	2012
78	151605661A	BBQ863	NF	NS	2013

“NF” indicates tags were not found in that location.
“NS” indicates that turtle was not sampled for genetic analysis.